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**FY 1990 ANNUAL REPORT**

**OCTOBER 1, 1989 THROUGH SEPTEMBER 30, 1990**

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## **INTRAMURAL RESEARCH**





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 22109-02 CMB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Comparison of Tissue Response to Complete Freund's and RIBI Adjuvant

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	J. A. Clark	Biol Lab Tech, VM	CMB, NIEHS
	J. R. Snipe	Biol Lab Tech, VM	CMB, NIEHS

## COOPERATING UNITS (if any)

Chemical Pathology Branch, DTRT, NIEHS  
Burroughs Wellcome

## LAB/BRANCH

Comparative Medicine Branch

## SECTION

Veterinary Medicine

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.1

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Complete Freund's adjuvant, a water in oil emulsion, is the most common adjuvant used to stimulate antibody response in rabbits. Its use is often associated with undesirable side effects at the inoculation site, such as inflammatory lesions, tissue necrosis, and even local sloughing. The RIBI Adjuvant System, an oil in water emulsion, is the most frequently used alternative to complete Freund's adjuvant. RIBI utilizes bacterial cell walls and byproducts which have been purified to eliminate the toxicity and allergenicity associated with the intact tubercle bacillus contained in complete Freund's adjuvant.

This study will examine intradermal, subcutaneous, intramuscular and intraperitoneal routes of inoculation in the rabbit, comparing the two adjuvants at varying dosage levels. Rabbits will be clinically evaluated for pain and distress, and gross and histopathologic collections will be made and examined at 1, 2, 3, or 4 weeks postinoculation. Rabbits scheduled for sacrifice on fertility studies will be used. Collaborations with investigators in other laboratories will be initiated to evaluate and compare antibody response to antigens under the varying experimental conditions. We hope to obtain a profile of the method(s) which result in maximum antibody response with minimum undesirable tissue reactions, benefitting the experimental animal and improving the scientific result.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES-22110-02 CMB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alopecia and Dermatitis in C57BL/6N Mice

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: J. E. Thigpen Head, Quality Assurance Lab CMB, NIEHS

Others: D. E. Blackmore Head, Veterinary Medicine CMB, NIEHS

M. F. Goelz Veterinary Medical Officer, VM CMB, NIEHS

G. F. Caviness Bio Lab Tech, QAL CMB, NIEHS

W. M. Yearby Bio Lab Tech, QAL CMB, NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Comparative Medicine Branch

## SECTION

Quality Assurance Laboratory

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

.1

## OTHER:

.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

C57BL/6N mice, an important strain in several NIEHS studies, develop an alopecia which usually arises at 4 to 6 months of age. This condition often progresses into a protracted dermatitis and may become severe enough that the animals develop ulcerative skin lesions and suffer premature morbidity and mortality. We have initiated a project directed at discovering a nutritional basis for the progressive skin disorders.

In a one-year pilot study, mice were divided into groups of 15 and fed either the standard NIH-31 diet (control diet) or a test diet made by fortifying the NIH-31 diet with vegetable oils, animal fat, thiamine, riboflavin, pyridoxine, cyanocobalamin, biotin, zinc oxide, or other vitamin and mineral mixtures. Preliminary indications are that obvious clinical differences are observed between groups and that the alopecia and degenerative skin conditions can be controlled by combinations of the vitamin and mineral mixtures. These preliminary findings suggest that C57BL/6N mice may require dietary changes as they age to compensate for natural degenerative skin changes that occur, probably due to strain dependent genetic predisposition.

A second study is underway to confirm these findings and hopefully provide additional information on the role of specific minerals/vitamins, etc., in preventing or inducing the alopecia, ulcerative skin lesions observed in C57BL/6N mice.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 Es 80001-18 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Microsomal Mixed-Function Oxidase System: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others:	D. Duignan	IRTA Fellow	LCMP	NIEHS
	E. Atta-Asafo-Adjei	Visiting Fellow	LCMP	NIEHS
	K. Nikbakht	Staff Fellow	LCMP	NIEHS

## COOPERATING UNITS (If any)

University of California, Davis, CA; Scripps Institute and Research Foundation;  
North Carolina State University

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Molecular Pharmacology Section

## INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

6.0

## PROFESSIONAL:

4.5

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three of the most highly expressed drug-metabolizing enzymes in rabbit lung are cytochrome P-450 isozymes IIB and IVB and the flavin-containing monooxygenase (FMO). Together these enzymes metabolize a wide variety of drugs, pesticides and other environmental chemicals. In addition to oxidation at carbon atoms, this group of enzymes also oxidizes sulfur, phosphorous and nitrogen. Drug-metabolizing enzymes may be involved in the activation of certain chemicals whose toxic effects are pulmonary specific. Because similar enzymes are also expressed in liver, the observation of tissue-specific effects suggests that different forms of the enzymes may be expressed in the two tissues. The liver/lung expression of P-450 IIB turns out to be quite complicated. Multiple forms of the enzyme having greater than 97% identity exist. However, one of the forms is expressed only in liver. This has been demonstrated by us in rabbit and hamster and by others in rat. This finding indicates that control of expression developed prior to speciation and that the structural portions of the different genes for IIB must undergo interconversion. In contrast to IIB, P-450 IVB may be encoded for by only a single gene. It is of interest that the 5'-flanking region of the rabbit IVB gene is not similar to the same region of the human gene. These differences may be important in the exclusion of IVB from human liver but not from rabbit liver. Previously, we have defined the differences between the structures of the FMO enzymes expressed in liver and lung. These enzymes, which are 56% identical, are clearly products of different genes. Hybrid proteins formed by combinations of the liver and lung cDNAs are expressed in COS cells but are inactive even though the secondary structures of the enzymes appear to be highly similar. Also, in examining some physical properties of the expressed FMO enzymes, we have noted that the liver enzyme does not retain the temperature sensitivity that it exhibits in hepatic microsomes. Presently, we are constructing a series of lung/liver and liver/liver chimerics in order to further investigate the catalytic properties of the FMO enzymes.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80031-14 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Altered Membrane Function in Xenobiotic Toxicity

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J.B. Pritchard	Research Physiologist	LCMP	NIEHS
Others:	D.S. Miller	Research Physiologist	LCMP	NIEHS
	R. Philpot	Research Chemist	LCMP	NIEHS
	D.A. Stewart	Staff Fellow	LCMP	NIEHS
	N. Wolff	Visiting Fellow	LCMP	NIEHS

## COOPERATING UNITS (if any)

University of Florida; Duke University

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Comparative Membrane Pharmacology Section

## INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

4.6

## PROFESSIONAL:

2.1

## OTHER:

2.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transport of solutes across epithelial membranes is a vital function of many organs, e.g., kidney, choroid plexus, liver and gut. Epithelial transport depends upon individual transport systems located in apical (BBM) and basolateral (BLM) membranes. Their complex organization, functional importance and exposed location make epithelial membranes particularly susceptible to toxic effects of foreign chemicals. Recent research has focussed on the mechanism and energetics of renal organic anion (OA) transport system, which determines how effectively many toxic xenobiotics are excreted from the body. Isolated BLM vesicles were used to demonstrate that OA entry was driven by the inwardly directed sodium gradient, but only in the presence of glutarate (GA) or  $\alpha$ -ketoglutarate ( $\alpha$ KG). This system appears to function through uptake of OA in exchange for internal GA or  $\alpha$ KG. The GA or  $\alpha$ KG is returned to the interior via sodium cotransport, maintaining the outwardly directed GA ( $\alpha$ KG) gradient needed to drive net OA accumulation. Thus, coupling to the sodium gradient is indirect, an example of *tertiary* active transport. Renal BBM vesicles from several species were used to show that exit of OA into the tubular lumen is much simpler, mediated by a carrier which can exchange internal OA for luminal anions, largely chloride or bicarbonate. Whole tissue preparations from rat and flounder kidney and crustacean urinary bladder have demonstrated the presence of GA( $\alpha$ KG)/Na driven OA transport across the BLM into the intact epithelium. Furthermore, recent studies using the fluorescent OA, fluorescein, has permitted direct demonstration of Na/GA-dependent OA transport across the intact renal epithelium of the flounder into the luminal compartment, i.e., that transepithelial transport is controlled by the basolateral uptake step as predicted by the earlier vesicle studies. Finally, as an initial step in assessing the control of the coupled system and biochemical characterization of the transporters involved in this system, renal m-RNA carrying the message for the OA system has been expressed in *Xenopus* oocytes.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80042-04 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Calcium Regulation and Signal Transduction Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.W. Putney, Jr.	Chief	LCMP	NIEHS
Others:	A. Hughes, F. Menniti, K. Oliver	Staff Fellows	LCMP	NIEHS
	M. Rossier, G. Bird	Visiting Fellows	LCMP	NIEHS
	K. Nogimori, G. Burgess	Guest Researchers	LCMP	NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Calcium Regulation Section

## INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

9.1

## PROFESSIONAL:

7.1

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The broad aim of this project is to understand at the cellular and molecular level the mechanisms by which surface membrane receptors for hormones, neurotransmitters and growth factors modify cellular responses through mobilization of cellular  $\text{Ca}^{2+}$ . An early event in the action of receptors of this class is the hydrolysis of a membrane lipid, phosphatidylinositol 4,5-bisphosphate to a  $\text{Ca}^{2+}$ -mobilizing second messengers, which releases  $\text{Ca}^{2+}$  from an intracellular organelle. The general approach in this project is to combine HPLC measurements of the formation and metabolism of inositol phosphates with real time measurements of cytosolic  $\text{Ca}^{2+}$  using intracellular fluorescent  $\text{Ca}^{2+}$  indicators. Our previous work has shown that a mechanism exists for signalling the entry of  $\text{Ca}^{2+}$  into cells following the depletion of  $\text{Ca}^{2+}$  from an intracellular organelle through the action of  $\text{IP}_3$ . We are currently attempting to understand the molecular mechanism underlying this process utilizing as a model that  $\text{Ca}^{2+}$ -mobilizing actions of the tumor promoter, thapsigargin, which activates this process while bypassing surface receptors and the generation of  $\text{IP}_3$ . We have also recently discovered a novel point of regulation through receptors of the metabolism of inositol phosphates, and are currently investigating the relevance of this step in various models of cellular signalling. Since the  $\text{Ca}^{2+}$  signalling system is centrally involved in the regulation of cellular growth under normal and pathological (neoplastic) conditions, these studies may provide novel perspectives on the pharmacological regulation and arrest of these processes. In addition,  $\text{Ca}^{2+}$  is believed to play a central role in mechanisms of chemically-induced cell injury, and thus these studies should provide insights into the mechanisms underlying the pathophysiological consequences of exposure to toxins and other environmental agents.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80043-03 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Channel Modulation by Signal Transduction Systems

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D.L. Armstrong	Senior Staff Fellow	LCMP	NIEHS
Others:	M. Austin	Biologist	LCMP	NIEHS
	A. Shcherbatko	Visiting Associate	LCMP	NIEHS
	R. White	Staff Fellow	LCMP	NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Calcium Regulation Section

## INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.5

## PROFESSIONAL:

2.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used patch clamp techniques to study the enzymatic regulation of voltage-activated calcium channels in a rat pituitary tumor cell line. Previous work in this laboratory has established that these dihydropyridine-sensitive calcium channels only respond to depolarization when the protein(s) forming the channel are phosphorylated; in the absence of phosphorylation they are completely inactivated. Furthermore, the response to depolarization is specific to the kinase that acts on the channel: protein kinase C does not alter channel activity but the cAMP-dependent kinase produces short bursts of very brief openings and a calcium/calmodulin-dependent kinase produces long bursts of much longer openings. Two phosphatases have also been implicated in the regulation of these channels. The calcium/calmodulin-dependent phosphatase 2b, calcineurin, rapidly inactivates the channels when intracellular calcium concentration exceeds  $1 \mu\text{M}$ . In addition, recent studies with okadaic acid, the potent tumor promoter that is the major toxin in shellfish poisoning, have also implicated phosphatase 1 in calcium channel regulation.

We are now beginning to characterize the role of these enzymes in calcium channel regulation dihydropyridines, compounds used clinically to treat stress-related human cardiovascular disease, and by hypothalamic neuropeptides that control pituitary hormone secretion. New evidence suggests that dihydropyridines produce their effects on these channels by altering their susceptibility to protein phosphorylation and its removal. In addition, we have shown that the GTP-dependent effects of neuropeptides reported widely in the literature do not result from direct interactions of GTP-binding proteins with the channels as suggested previously. Instead, the G proteins appear activate the kinases and phosphatases through conventional second messengers. These experiments are leading us to a molecular model of calcium channel function that will contribute to an understanding of the toxicity of environmental hazards.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80044-02 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Embryonic Neural Induction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D. Armstrong	Senior Staff Fellow	LCMP NIEHS
	D. Miller	Research Physiologist	LCMP NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Calcium Regulation Section and Comparative Pharmacology Section

## INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.2

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In some of the most famous experiments in embryology, Spemann and Mangold demonstrated the potential for brain formation is not restricted to specific cells in the ectoderm of early amphibian embryos, but is induced by cell contact with the underlying chordamesoderm. The central concept which emerged from their work, of cell interactions determining cell fate, remains a cornerstone of modern embryology. Nevertheless, over fifty years later, both the molecular signal that induces neural differentiation and the method of its communication remain to be discovered. We have begun to reinvestigate the problem of neural induction with modern microinjection techniques in embryonic ascidians, primitive marine chordates with a simple, archetypal development. We have studied several local species of ascidians with a view to obtaining the most amenable preparation for our studies. We have chosen *Ascidia interrupta* for its relatively large and transparent eggs. These hermaphrodites produce fertile eggs and sperm throughout the year, and they self-fertilize with reasonable efficiency. Thus, control and experimental protocols can be carried out on a clutch of genetically identical embryos. Furthermore, the block to polyspermy does not reside in the extraembryonic membranes, so we have removed them enzymatically without disrupting development, which proceeds to the tadpole stage in less than 18 hours at room temperature. Microelectrodes will be used to inject specific pharmacological probes into single, identified blastomeres. The effect of these compounds on neuronal development will be determined by simultaneously filling the blastomeres in the presumptive neuroectoderm with fluorescent tracers of cell lineage (conjugated dextrans) and of cell coupling across gap junctions (Lucifer yellow). These experiments are designed to illuminate one of the most fundamental unsolved problems in biology: How does one cell alter the fate of its neighbor during vertebrate embryogenesis?





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 Es 80045-02 LCMP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GTP-Binding Proteins and Signal Transduction: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	M. Rodbell	Senior Research Scientist	LCMP	NIEHS
Others:	F. Ribeiro-Neto	Visiting Associate	LCMP	NIEHS
	K. Haraguchi	Visiting Associate	LCMP	NIEHS
	D. Udrisar	Visiting Fellow	LCMP	NIEHS

COOPERATING UNITS (if any)

Rocky Mt. Laboratory, MT, National Institute of Allergy and Infections Diseases

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Signal Transduction Section

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

G-proteins (GTP binding regulatory proteins) are the transducing elements that transmit the actions of hormones, neurotransmitters, odorants, light, antigens, and a variety of chemical signals that interact with receptors on the external surfaces of all eukaryotic cells, from yeast to man. Based on actions of cross-linking agents and hydrodynamic properties, these proteins isolated from rat brain synaptosomes behave as multimeric, polydisperse structures. They resemble in their temperature sensitivities and disaggregating effects of guanine nucleotides cytoskeletal proteins such as actin and tubulin. Four of the prominent G-proteins found in brain display similar but varying properties depending on the species. Assuming these findings with extracted materials apply to intact cells, a new concept of signal transduction has been developed. In a related study, 100 kDa GTP-binding proteins containing epitopes reactive with antibodies against classical transduction proteins have been discovered in the liver and characterized. Finally, hormones acting through G proteins have been shown to regulate fluid phase endocytosis in rat adipocytes and in Chinese Hamster Cells transfected with genes for muscarinic receptors I and III. These studies reveal a new function of G-proteins and suggest that the processing of pinosomes may be an important route for the dissemination of environmental signals and adaptation to the constantly changing levels of these signals in the environment.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80046-02 LCMP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Inositol Lipid Signalling Mechanisms

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	S.B. Shears	Visiting Scientist	LCMP	NIHS
Others:	P.J. Hughes	Visiting Fellow	LCMP	NIHS
	M. Abdullah	Senior Staff Fellow	LCMP	NIHS

## COOPERATING UNITS (if any)

University of East Anglia, U.K.

## LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

## SECTION

Inositol Lipid Section

## INSTITUTE AND LOCATION

NIHS/NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.4

## PROFESSIONAL:

1.2

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activation of cell-surface receptors releases inside cells inositol (1,4,5) trisphosphate ( $I(1,4,5)P_3$ ), a ubiquitous signal playing a pivotal role in cell regulation through initiation of calcium fluxes. Enzymes that metabolize and thereby deactivate  $I(1,4,5)P_3$  are crucial to the regulation of cell signalling. Moreover, increasing evidence points to the ensuing metabolites themselves having important roles in signal transduction. Particular attention is being focused on  $IP_5$  and  $IP_6$ , since data from this laboratory, in collaboration with the Calcium Regulation Section of LCMP, has shown a novel consequence of receptor occupation to be a stimulation of  $IP_5$  breakdown to  $I(3,4,5,6)P_4$ . This project aims to understand how metabolism of inositol phosphates is regulated by extracellular agents such as hormones, toxins (including carcinogens) and clinically important drugs; inositol phosphate fluxes in isolated cells and cell lines, and the influence of extracellular agents, will be monitored to seek possible control points. Complementary techniques are either in use or being developed: (a) isolation of enzymes from cell extracts in both unregulated and regulated states, (b) reconstitution of purified enzymes with  $Ca^{2+}$ /calmodulin, A-kinase and C-kinase and (c) cloning and expressing key enzymes in cell-lines to study physiological effects. Growing evidence (largely from this laboratory) also points to feedforward and feedback regulation by inositol phosphates themselves. As the complexities of this system are unravelled, we will better understand and treat the effects of extracellular toxins on cell signalling.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 80047-01 LCMP

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intracellular Receptors and Metabolic Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D.S. Miller Research Physiologist LCMP NIEHS

COOPERATING UNITS (if any)

Michigan Cancer Foundation; Weizmann Institute

LAB/BRANCH

Laboratory of Cellular and Molecular Pharmacology

SECTION

Intracellular Regulation Section

INSTITUTE AND LOCATION

NIEHS/NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.75

PROFESSIONAL:

0.75

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Complex organisms use chemical signals to integrate and coordinate the function of specialized tissues. In many cases, information is transferred in the form of chemicals, e.g., polypeptide hormones or growth factors, which interact with target cells through specific, surface receptors and profoundly affect cell structure and function. This project is concerned with the question of how such information flows to intracellular metabolic control points, i.e., defining postreceptor signalling mechanisms. Recent research has focussed on the role of intracellular receptors in the overall mechanism of insulin action. For these studies, we have developed a powerful experimental system consisting of a giant, insulin-sensitive cell (amphibian oocyte), paraffin oil-based cell microinjection and fractionation procedures and single cell microanalysis. Using this system, we have obtained the first evidence that intracellular insulin can directly affect cell metabolism. That is, microinjected, intracellular insulin stimulates both transcription and translation by acting at nuclear and cytoplasmic sites. Such actions are independent of the surface receptor, since they also occur in isolated nuclei and cytoplasm samples. Insulin action at internal sites also occurs when cells are exposed to extracellular hormone, since the insulin-stimulated component of protein synthesis is partially blocked when cells are first exposed to external hormone and then microinjected with antibody. Inhibition by antibody is only found in cells which have accumulated undegraded insulin intracellularly.

Future plans include 1) defining the mechanisms by which insulin gains access to the internal receptors and through them affects metabolism, 2) determining the extent to which intracellular receptors are involved in the action polypeptide hormones and growth factors in somatic cells, and 3) assessing the role of disrupted intracellular signalling in pathological states, e.g., insulin resistance.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 60099-11 LG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Organization-regulation of mammalian lactate dehydrogenase genes

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others:	M. Maekawa	Special Volunteer	LG, NIEHS
	K. Sudo	Special Volunteer	LG, NIEHS

## COOPERATING UNITS (if any)

Dr. Kanno, Hamamatsu University School of Medicine, Hamamatsu City, Japan;  
Drs. Ikawa and Machida, Jikei University School of Medicine, Tokyo, Japan;  
Dr. Kitamura, Ogata Institute for Medical and Chemical Research, Tokyo, Japan

## LAB/BRANCH

Laboratory of Genetics

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

.3

## PROFESSIONAL:

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- |   |   |                                      |
|---|---|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |   |                                      |
| <input type="checkbox"/> (a2) Interviews    |   |                                      |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular structure of human LDH-A and LDH-B mutant genes have been characterized.

Human lactate dehydrogenase-A mutant gene was isolated from the genomic DNA library of a patient deficient in LDH-A (muscle) subunit. The nucleotide sequences of seven protein-coding exons were determined and a deletion of 20 base-pairs in exon 6 was found. This mutation results in a frameshift translation and premature termination. The predicted incomplete LDH-A (M) subunit containing only 259 instead of 331 amino acids appears to be degraded rapidly, since no protein was detected immunologically.

A human lactate dehydrogenase-B mutant gene was isolated from a genomic DNA library constructed from a patient with unstable LDH-B (heart) subunit. The nucleotide sequences of seven protein-coding exons were determined and a single nucleotide substitution of G by A at Arg codon CGC in exon 4 was found. This mutation results in an amino-acid replacement of a conserved arginine by histidine at the residue "173," which is involved in an anion-binding site at the P-axis of LDH subunits.

This information will allow more accurate evaluation of genetic mutation events caused by mutagens and eventually will be of value to improve human health care.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 ES 61019-10 LG
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Collaborative protein sequencing and peptide synthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Steven S.-L. Li	Research Geneticist	LG, NIEHS
Others: Farida S. Sharief Hitoshi Miyasaka Jun M. Versola	Biologist Visiting Fellow Biological Aid (SIS)	LG, NIEHS LG, NIEHS LG, NIEHS
COOPERATING UNITS (if any) Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.3	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)  <p>The hydrophilicity of human prostatic and lysosomal acid phosphatases were analyzed and the relative antigenicity (capacity to bind antibodies raised against the intact prostatic acid phosphatase) of the selected peptides was evaluated in a competitive assay. Both prostatic and lysosomal acid phosphatases were shown to possess similar antigenic structure on both terminal regions, along with more similarity on NH<sub>2</sub>-terminal peptide than COOH-terminal site. At least one additional antigenic site is present at the internal region of prostatic acid phosphatase, since the mixture of both amino- and carboxyl-terminal peptides exhibited only 70% inhibition.</p> <p>The collaborative protein chemistry laboratory with UNC has already provided lots of research services on protein microsequencing and peptide synthesis to other scientists at the NIEHS.</p>		







## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61032-07 LG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure-function of mammalian lactate dehydrogenase isozymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: Steven S.-L. Li

Research Geneticist

LG, NIEHS

Others: Soichi Tsuji

Special Volunteer

LG, NIEHS

## COOPERATING UNITS (if any)

Dr. Yang, Department of Cell Biology, Baylor College of Medicine, Houston, TX;  
Dr. W.-H. Li, Center for Demographic and Population Genetics, University of  
Texas Health Sciences Center, Houston, TX

## LAB/BRANCH

Laboratory of Genetics

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary structure of 333 amino acids from mouse and rat LDH-B<sub>4</sub> (heart) are being determined by sequencing both protein and cDNA. A comparison between mouse and human LDH-B sequence revealed eight (2.4%) amino acid differences: four differences are clustered within the random-coil region of amino-terminal 20 residues, two substitutions at residue numbers 52 and 132 are located in the  $\beta$ -sheet, and two changes at residue numbers 236 and 317 are positioned in  $\alpha$ -helix.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 ES 65021-18 LG
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Investigation of Germinal Mutation Induction in Mice		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	F. M. Johnson	Research Geneticist LG, NIEHS
Others:	M. L. Snell	Biologist LG, NIEHS
COOPERATING UNITS (if any) Dr. S. E. Lewis, Research Triangle Institute, Life Sciences Group, Research Triangle Park, N.C.; Dr. D. P. Lovell, British Industrial Biological Research Association, Carshalton, Surrey, England		
LAB/BRANCH Laboratory of Genetics		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>The objectives of this project are (1) to detect natural and induced mutations in mice, (2) to achieve understanding of the molecular events occurring in the process of mutation induction, and (3) to relate these events to the life, form and function of the mammalian organism. Our project is relevant to the problem of human exposures to environmental chemicals; particularly, the increased risk of genetic disease in the offspring of exposed individuals. We have detected a variety of mutations at specific biochemical loci with electrophoretic methods, characterized several normal and mutant genes (and gene products), and examined the offspring of mutagen-treated and control parents for the physical manifestation of polygenic mutations affecting the skeleton.</p> <p>Recently, we identified a group of highly variable animals produced from ethylnitrosourea-treated males. We analyzed the variability to distinguish abnormalities of both size and shape. The experiment involved over 300,000 quantitative measurements on 12 bones from about 1,000 mice. The animals showing the effect were produced immediately after the male parents recovered from a period of sterility induced by the chemical. All twelve of the bones examined were affected. The progeny from matings that took place before the sterile period were normal. The progeny from much later matings were also normal. Virtually all offspring in the one treatment group were affected, and the expression of the variability was different in each individual.</p> <p>The results suggest that children may show abnormal growth and development under conditions of parental male exposure like those in our experiment. Although we studied only the skeleton, it would be reasonable to suspect that other structures and functions were also damaged.</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 ES 65033-07 LG
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>In Vivo Mammalian Mutagenesis</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:           H. V. Mallings J. G. Burkhardt	Research Geneticist Research Chemist	LG, NIEHS LG, NIEHS
Others:   K. S. Sampson J. L. Bradley	Biological Aid (SIS) Summer IRTA	LG, NIEHS LG, NIEHS
COOPERATING UNITS (if any) C. A. Hutchinson, III & M. H. Edgell, UNC, Chapel Hill, N. C. E. J. Eisen, NCSU, Raleigh, N. C. Clement Markert, NCSU, Raleigh, N. C.		
LAB/BRANCH <b>Laboratory of Genetics</b>		
SECTION		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS: 2.75	PROFESSIONAL: 1.0	OTHER: 1.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The objective of this research is to study mutagenesis at the DNA level in mammals and to evaluate genetic and biochemical events in certain mutants as models of human genetic diseases. A major problem in mutagenesis is that the level and specificity of response is very different between indicator organisms; predictions about induced mutagenesis may not be relevant. Significant variation is due to the diversity of the marker genes; a single sequence needs to be used among various organisms and tissues. Our analyses are based on reverse mutations among single copies of the <math>\Phi</math>X174 virus as a shuttle vector in different species. The accomplishments are as follows. 1) Experiments with transgenic cell cultures and <math>\Phi</math>X174am3cs70 indicate that the approach is sensitive enough to study mutations among single copies of the vector DNA recovered from the host. 2) Transgenic inbred C57Bl/6 mice containing the <math>\Phi</math>X vector have been produced and the transgene has been transmitted to offspring. Each hemizygous founder contains more than 50 copies per genome. Mating schemes have expanded the number of copies per genome and produced mice homozygous for the vector at each allelic insertion site. 3) Methods have been developed to efficiently recover the vector from the transgenic mice and measurements of mutation rates are in progress. 4) A second type of transgenic <math>\Phi</math>X vector with a different mutation site and reversion mechanism has been developed. Production of transgenic mice with this new vector is in progress. The use of integrated transgenic vector can combine a theoretical study of mutations in several model organisms with the assessment of mutagenic hazard. A single DNA sequence can be exposed and analyzed as naked DNA, as a single stranded virus, double stranded in bacteria, and in the nuclear genome of mammalian cells or transgenic mice. In addition, such an approach may allow us to examine <u>in vivo</u> mutagenesis and repair in many somatic tissues and in gametogenic tissue during development or as a function of aging and various conditions of environmental exposure.           </p>		





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 10004-11 LMB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>NMR Studies of the Mechanisms of Cell Injury</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robert E. London	Research Physicist      LMB    NIEHS
Other:	Elizabeth Murphy	Research Physiologist      LMB    NIEHS
	Louis A. Levy	Research Chemist      LMB    NIEHS
COOPERATING UNITS (if any) Professor Charles Steenberg, Department of Pathology, Duke University, Durham, NC; Prof. Leonard S. Gettes, Dept. of Medicine, UNC Medical School, Chapel Hill, NC.		
LAB/BRANCH <b>Laboratory of Molecular Biophysics</b>		
SECTION <b>Nuclear Magnetic Resonance Group</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           This research is focused on the role of ionic alterations in the development of irreversible cell injury. We make use of <i>vivo</i> and <i>in situ</i> NMR methods on cell suspensions and perfused organs in order to determine the cytosolic concentrations of these ions and to monitor associated metabolic changes to which the NMR technique is sensitive. We have previously demonstrated that increases in cytosolic calcium (<math>Ca_i</math>), sodium (<math>Na_i</math>), magnesium (<math>Mg_i</math>), and hydrogen and a decrease in ATP occur during cell injury. Although a consensus appears to be developing that an increase in <math>Ca_i</math> precedes the onset of irreversible injury, this does not prove that an increase in <math>Ca_i</math> is necessary or responsible for the onset of irreversible cell injury. We therefore undertook studies to determine whether it was possible to manipulate experimental conditions to prevent or delay an increase in <math>Ca_i</math>. The perfused rat heart was studied under conditions of: total ischemia, potassium arrest, magnesium arrest, and pretreatment with 0.9 <math>\mu M</math> diltiazem to reduce but not abolish contractility. In all conditions tested, the increase in <math>Ca_i</math> preceded lethal injury, as determined by enzyme release. Thus, we have not been able to dissociate the increase in calcium from irreversible cell injury.         </p> <p>           Using the NMR sensitive indicator 5FBAPTA to measure <math>Ca_i</math>, we have also demonstrated that amiloride significantly attenuates the increase in <math>Ca_i</math> observed during ischemia. In addition, since ATP, measured by <math>^{31}P</math> NMR, falls with a similar time course in the presence and absence of amiloride, we can therefore dissociate the increase in <math>Ca_i</math> from the decrease in ATP. We observed that the presence of amiloride during ischemia allows better recovery of contractile function during reflow after 20 minutes of ischemia. These data strongly suggest that the rise in <math>Ca_i</math> plays an important role in the contractile dysfunction which occurs after ischemia.         </p>		





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 30003-19 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Biochemical Methodology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Phillip W. Albro

Research Chemist

LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Metabolism

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.3

## OTHER:

0.7

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We had previously developed techniques for administering test chemicals to earthworms by gavage, and for collecting respiratory carbon dioxide from these animals. More recently we have improved these techniques such that it is possible to accurately determine the dosages actually administered, and to avoid contamination of the trapped carbon dioxide by other (radiolabeled) volatile compounds. We are in the process of developing a method for using fluorinated analogs of radiolabeled test chemicals to elucidate metabolic pathways in which the first step is highly rate-limiting (so that no intermediates accumulate for identification). The oxidation of o-phthalic acid to CO<sub>2</sub> by earthworms appears to be such a pathway. Through the use of 3-, 4- and poly substituted analogues along with radiolabeled, unsubstituted phthalic acid we are able to deduce the sites of oxidative attack on the ring carbon atoms.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 ES 50046-12 LMB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Mechanisms of Chemically Induced Photosensitivity</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Colin F. Chignell      Chief, LMB Piotr Bilski          Visiting Fellow Krzysztof Reszka      Visiting Scientist Anson S.W. Li          Staff Specialist	LMB    NIEHS LMB    NIEHS LMB    NIEHS LMB    NIEHS
Other	Robert H. Sik          Biologist	LMB    NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Laboratory of Molecular Biophysics</b>		
SECTION <b>Molecular Biophysics</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS: <b>3.2</b>	PROFESSIONAL <b>2.7</b>	OTHER: <b>0.5</b>
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>           Light is known to interact with endogenous or exogenous chemical agents in the skin or eyes, to produce photosensitization (phototoxicity or photoallergy). The objective of this project is to determine whether light-induced free radicals play a role in photosensitization. Electron spin resonance studies have shown that UV-irradiation of the anti-psoriatic drug anthralin (AN) resulted in the generation of the superoxide anion radical (<math>O_2^{\cdot -}</math>), which was identified by spin trapping with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). In the absence of oxygen, the drug abstracted hydrogen atoms from the solvent ethanol. However, 1,8-dihydroxyanthraquinone (1,8-DHAQ), the major AN photoproduct, was much more active than AN itself in generating superoxide and ethanol radicals. This suggests that AN photosensitization may be due to 1,8-DHAQ and not AN. Disperse blue 35 is an anthraquinone-based dye mixture which causes photocontact dermatitis in factory workers. The main component of the dye, 4,5-diamino-1,8-dihydroxyanthraquinone, was found to be a potent generator of both singlet oxygen (<math>^1O_2</math>) and <math>O_2^{\cdot -}</math> upon visible light irradiation. UV-irradiation of another photosensitizing anthraquinone-derived dye benzanthrone (7H-benz[de]anthracen-7-one) resulted in the generation of both <math>^1O_2</math> and <math>O_2^{\cdot -}</math> in high yield. Active forms of oxygen were also implicated in the photo-killing of gram-positive bacteria by curcumin. In contrast halogenated aromatic photosensitizers (e.g. amiodarone, bithionol, salicylanilides, chlorpromazine) undergo dehalogenation upon UV-irradiation to yield the corresponding aryl radicals which may undergo hydrogen-abstraction reactions <i>in vivo</i>.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50080-08 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Health Applications of Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Kenneth B. Tomer Research Chemist LMB NIEHS

Other: Carol E. Parker Chemist LMB NIEHS

Steven McGown Chemist LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

One of the components of the mass spectrometry workgroup mission is to provide other groups within NIEHS access to mass spectrometric analyses on a service basis. The workgroup provides the following services on an ongoing basis: 1) low and high resolution electron impact (EI) mass spectra; 2) low and high resolution chemical ionization (CI) mass spectra; 3) negative ion chemical ionization (NICI) mass spectra; 4) gas chromatography/mass spectrometry (GC/MS) in conjunction with EI, CI and NICI mass spectra; 4) gas chromatography/mass spectrometry (GC/MS) in conjunction with EI, CI and NICI MS; 5) thermospray (TSP) liquid-chromatography/mass spectrometry (LC/MS) in conjunction with CI and NICI MS; 6) electro-spray ionization; 7) electro-spray ionization; 8) fast atom bombardment (FAB) under both positive and negative ion conditions; 8) continuous flow FAB/MS and FAB/MS/MS under both positive and negative ion conditions; and 10) tandem MS in combination with positive and negative ion FAB, EI and CI MS.

During the past year approximately 630 MS analyses have been performed on a service basis (not including collaborative work).

In addition to mass spectrometric services provided to other NIEHS scientists, we have been called upon to provide high performance liquid chromatographic (HPLC) support. With the addition of capillary zone electrophoresis (CZE) capabilities in this lab, these service aspects are expected to increase in importance.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50082-07 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Tumor Promoters and Antipromoters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Phillip W. Albro

Research Physicist

LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Metabolism

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been discontinued.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50087-04 LMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Singlet Oxygen-Dependent Photosensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Reza Dabestani Staff Fellow LMB NIEHS  
Colin F. Chignell Chief, LMB LMB NIEHS

Other: Robert H. Sik Biologist LMB NIEHS

COOPERATING UNITS (if any)

Dr. Ann G. Motten, Duke University, Durham, N.C.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and eyes. This process can produce undesirable clinical consequences, as in phototoxicity and photoallergy; or it can have beneficial effects, as in tumor photodynamic therapy (PDT) and coal-tar or psoralen (PUVA) therapy against psoriasis. Photosensitization results from the light-induced production of free radicals and/or singlet oxygen ( $^1O_2$ ), the lowest electronic excited state of molecular oxygen. Because the latter species may be important in both phototoxic reactions and PDT, we have developed state-of-the-art instrumentation capable of detecting the characteristic phosphorescence of  $^1O_2$  at 1268 nm. This instrumentation has permitted us to delineate the photophysics of  $^1O_2$  production from a number of photosensitizers including phenothiazines, tetracyclines, benzoxazoles synthetic dyes, anthralin and 1,8-dihydroxyanthraquinone. The major component of Disperse blue 35 (a dye that causes photodermatitis in factory workers) was identified as 4,5-diamino-1,8-dihydroxyanthraquinone and shown to be an efficient generator of  $^1O_2$ . Singlet oxygen was also implicated in the phototoxicity of benzanthrone (7H-benz[de]anthracene-7-one), a dye intermediate prepared from 1,8-dihydroxyanthraquinone. A nano-second flash photolysis spectrometer has been built and is being tested. This equipment will permit us to carry out time-resolved transient, absorption and emission spectroscopy on excited state intermediates (precursors to  $^1O_2$ ) of photosensitizers.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50088-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relationship of Free Radicals to Halocarbon-Induced Toxicity in the Liver

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald P. Mason Research Chemist LMB NIEHS

Other: Kathy T. Knecht Guest Worker LMB NIEHS  
 Henry D. Connor Research Chemist LMB NIEHS  
 David Duling Programmer/Analyst CSC NIEHS

## COOPERATING UNITS (if any)

Dr. Ronald G. Thurman, Department of Pharmacology, UNC, Chapel Hill, NC

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Electron Spin Resonance

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.4

## PROFESSIONAL:

1.2

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

$\text{CCl}_4$  has been shown previously to be metabolized to the trichloromethyl radical ( $\cdot\text{CCl}_3$ ) and to a novel oxygen-containing carbon dioxide anion radical ( $\cdot\text{CO}_2^-$ ) in the perfused rat liver. The  $\cdot\text{CO}_2^-$  radical adduct also was observed in urine following the intragastric administration of  $\text{CCl}_4$  or  $\text{CBrCl}_3$  and spin trap. The rate of formation of  $\cdot\text{CO}_2^-$  radical adduct was decreased 2-3 fold following inhibition of cytochrome P-450-dependent mono-oxygenases by metyrapone (0.5 mM) and was increased about two-fold by induction of cytochrome P-450 by phenobarbital pretreatment. Toxicity of halocarbons in the perfused liver was assessed by measuring the release of lactate dehydrogenase (LDH) into the effluent perfusate in livers from phenobarbital-treated rats under conditions identical to those employed to detect radical adducts. Metabolism of halocarbons to the  $\cdot\text{CO}_2^-$  radical adduct was 6-8 fold faster during perfusion with nitrogen-saturated rather than with oxygen-saturated perfusate. Concomitantly, liver damage detected from LDH release occurred much sooner during halocarbon infusion in the presence of nitrogen-saturated perfusate. A good correlation ( $r = -0.8$ ) between the rate of formation of  $\text{PBN}/\cdot\text{CO}_2^-$  and the time to onset of LDH release following halocarbon infusion was observed. Therefore, it is concluded that  $\text{PBN}/\cdot\text{CO}_2^-$  is a useful marker for the free radical intermediates that are casually related to halocarbon-induced hepatotoxicity. Recently, the  $\cdot\text{CCl}_3$  and  $\cdot\text{CO}_2^-$  radical adducts also have been detected in the bile from anesthetized rats. Hypoxia or pretreatment with phenobarbital has been reported to enhance the hepatotoxicity of  $\text{CCl}_4$  *in vivo*; these treatments also produced an increase in the biliary concentration of the  $\text{PBN}/\cdot\text{CCl}_3$  radical adduct and in the  $\cdot\text{CCl}_3$ -derived  $\text{PBN}/\cdot\text{CO}_2^-$  radical adduct as well. ESR analysis of bile from animals treated with free radical traps and other xenobiotics, such as ethanol, may prove useful in monitoring hepatic free radical-adduct formation *in vivo*.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50089-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Reaction of Free Radical Metabolites with DNA

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
Other:	Mark Burkitt	Visiting Fellow	LMB	NIEHS
	David Duling	Programmer/Analyst	LMB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Electron Spin Resonance

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.4

## PROFESSIONAL:

1.2

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The interaction of free radical metabolites with DNA has been a major area of interest and speculation, but previous electron spin resonance (ESR) investigations of this area have been very limited. The reaction of the hydroxyl radical, generated by a Fenton system, with pyrimidine deoxyribonucleotides was investigated using the ESR technique of spin trapping. The spin trap *t*-nitrosobutane was employed to trap secondary radicals formed by the reaction of the hydroxyl radical with these nucleotides. The results presented here show the hydroxyl radical attack on thymidine, 2-deoxycytidine 5-monophosphate and 2-deoxyuridine 5-monophosphate produced nucleotide-derived free radicals. The results indicate that  $\cdot\text{OH}$  radical attack occurs predominantly at the carbon-carbon double bond of the pyrimidine base. The ESR studies showed a good correlation with previous work produced by authors who used x- or  $\gamma$ -ray irradiation to generate the hydroxyl radical. A thiobarbituric acid assay was also used to monitor the damage produced to the nucleotides by the Fenton system. These results showed qualitative agreement with the spin trapping studies.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50090-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Porphyrin Ion Radical Metabolites and Their Reactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald P. Mason Research Chemist LMB NIEHS

Other: Herbert Sipe Research Chemist LMB NIEHS

David Duling Programmer/analyst LMB NIEHS

## COOPERATING UNITS (# any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Electron Spin Resonance

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Uroporphyrin I, which accumulates in body tissues of congenital erythropoietic porphyria patients, can undergo an enzymatic one-electron reduction to the porphyrin anion radical when a suitable reducing cofactor is present. We have demonstrated that anaerobic microsomal incubations containing NADPH and uroporphyrin I give an electron spin resonance spectrum of a porphyrin anion free radical. Inhibitor studies indicate that NADPH-cytochrome P-450 reductase is the electron donor. This radical undergoes a second-order decay due to nonenzymatic disproportionation of the radical. Aerobic microsomal incubations were also investigated for the reduction of oxygen to superoxide by monitoring oxygen consumption and the spin-trapping of superoxide. These experiments demonstrated that electron transfer from the porphyrin radical to molecular oxygen does occur, but due to the slow formation of the radical anion, no oxygen consumption above the basal level could be detected in the microsomal incubations. The photoreduction of uroporphyrin I in aerobic and anaerobic incubations was also investigated. Similar results have been obtained with photofrin II, a photo-activated antitumor agent. The oxidation of a variety of porphyrins to cation free radicals by peroxidases also has been investigated. Since the enzyme intermediate of horseradish peroxidase, compound I, is itself a porphyrin IX cation radical, this work will have implications for electron transfer as well as porphyrin metabolism.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50091-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenyl Radical Formation by Oxyhemoglobin from Phenylhydrazine *In Vivo*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Ronald P. Mason	Research Chemist	LMB	NIEHS
Other:	Christopher Kennedy	NRC Fellow	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	CSC	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Electron Spin Resonance

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

0.7

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The reaction of oxyhemoglobin with phenylhydrazine has received considerable attention for many decades. The basis for this interest stems from the ability of phenylhydrazine and hydrazine-based drugs to induce hemolytic anemia. Considerable evidence obtained from *in vitro* electron spin resonance (ESR) experiments implicates free radicals in the events leading to red blood cell hemolysis. However, until this report, no corroborating ESR evidence for *in vivo* free radical formation has been presented. We have successfully employed ESR to detect the formation of a radical adduct in the blood of rats which received an intragastric dose of phenylhydrazine followed by an intraperitoneal injection of the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO). The results of a series of experiments with sulfhydryl reagents and C-13-labelled phenylhydrazine led us to assign this DMPO radical adduct to the trapping of a hemoglobin-derived thiyl free radical. In addition to phenylhydrazine the hydrazine-based drugs isoniazid, iproniazid, phenelzine, and hydralazine were examined. Of the four drugs, only phenelzine and iproniazid were able to induce the formation of the DMPO/hemoglobin thiyl free radical adduct *in vivo*, whereas only phenelzine and hydralazine were able to form this adduct *in vitro*. We were able to decrease the *in vivo* iproniazid-induced adduct formation by pretreating the rats with bis-*para*-nitrophenylphosphate, an arylamidase inhibitor. Our results support the idea that iproniazid is hydrolyzed in the liver to a more reactive metabolite, most likely isopropylhydrazine, which is subsequently released into the blood stream. DMPO/hemoglobin thiyl free radical formation is not limited to hydrazines, but forms when either hydroperoxides or aromatic hydroxylamines react with oxyhemoglobin. This species may be *in vivo* indicator of free radical damage to red blood cells.



## DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50096-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Changes in Tissue Non-Cyclic Phosphodiesterases Produced by Toxins

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Tyler Burt Expert LMB NIEHS

Other: Robert E. London Research Physiologist LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Nuclear Magnetic Resonance Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been discontinued.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50101-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Tetrachlorodibenzofuran Metabolites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Kenneth B. Tomer Research Chemist LMB NIEHS

Other: Steven R. McGown Chemist LMB NIEHS  
L.T. Burka Chemist DTRT/STB NIEHS

## COOPERATING UNITS (if any)

DTRT/STB

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been combined with Z01 ES 50080-07 LMB.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50103-04 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GC-MS Analysis of PCDF Blood Levels in Children Exposed *In Vitro*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Walter Rogan	Chief, Epidemiology Branch	DBRA/EB	NIEHS
Other:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS

## COOPERATING UNITS (if any)

Dr. Linda Sheldon, RTI, Research Triangle Park, NC

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0

## PROFESSIONAL:

0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been combined with Z01 ES 50108-02 LMB and Z01 ES 50080-08 LMB.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 50104-04 LMB</b>															
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>In Vivo Studies of Cellular Magnesium</b>																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 40%;">Robert E. London</td> <td style="width: 20%;">Research Physicist</td> <td style="width: 10%;">LMB</td> <td style="width: 10%;">NIEHS</td> </tr> <tr> <td>Other:</td> <td>Elizabeth Murphy</td> <td>Research Physiologist</td> <td>LMB</td> <td>NIEHS</td> </tr> <tr> <td></td> <td>Louis A. Levy</td> <td>Research Chemist</td> <td>LMB</td> <td>NIEHS</td> </tr> </table>			PI:	Robert E. London	Research Physicist	LMB	NIEHS	Other:	Elizabeth Murphy	Research Physiologist	LMB	NIEHS		Louis A. Levy	Research Chemist	LMB	NIEHS
PI:	Robert E. London	Research Physicist	LMB	NIEHS													
Other:	Elizabeth Murphy	Research Physiologist	LMB	NIEHS													
	Louis A. Levy	Research Chemist	LMB	NIEHS													
COOPERATING UNITS (if any) <b>Professor Melvyn Lieberman, Division of Physiology, Department of Cell Biology, Duke University Medical Center, Durham, N.C.</b>																	
LAB/BRANCH <b>Laboratory of Molecular Biophysics</b>																	
SECTION <b>Nuclear Magnetic Resonance Group</b>																	
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>																	
TOTAL MAN-YEARS: <b>2.1</b>	PROFESSIONAL: <b>1.5</b>	OTHER: <b>0.6</b>															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews								
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither															
<input type="checkbox"/> (a1) Minors																	
<input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Although magnesium is the most abundant divalent metal ion found in most cells, the regulation of the cytosolic free magnesium ion concentration (<math>Mg_i</math>), and the role which alterations in the level of this ion may play in mediating the pathology of a variety of toxins, is relatively unknown. In order to attack this problem, we have previously designed and synthesized both NMR and fluorescent indicators which can be loaded into cells and are sensitive to the free magnesium ion level. Over the past year we have applied these indicators to the study of mechanisms which regulate <math>Mg_i</math>. In order to understand how alterations in <math>Mg_i</math> might be involved in cell injury, it is important to understand the normal physiological regulation of <math>Mg_i</math>. We varied the ionic composition of the medium in which the cells were perfused and obtained evidence for an indirect effect reflecting competition between intracellular calcium and magnesium ions for binding sites. To understand the mechanisms involved in modulating <math>Mg_i</math>, we examined the effect of altering cytosolic pH. Cultured embryonic chick heart cells were grown on coverslips and loaded with either the fluorescent <math>Mg_i</math> indicator FURAPTRA, or the pH indicator BCECF. Internal pH was altered by exposing the cells to 10 mM <math>NH_4Cl</math>. When neutral <math>NH_3</math> crossed the membrane, the intracellular pH rose transiently to ~ 7.9; this was accompanied by a 0.18 mM decrease in <math>Mg_i</math>. After a few minutes, pH<sub>i</sub> recovered to 7.25 and <math>Mg_i</math> returned to control levels. Upon removal of extracellular <math>NH_4Cl</math>, intracellular pH fell to 6.5, whereas <math>Mg_i</math> increased by only 0.06 mM. Total cell magnesium measured by atomic absorption spectroscopy was not altered by these manipulations. Although the dissociation constant of calcium ions from FURAPTRA of 53 <math>\mu M</math> is well above basal cytosolic calcium levels, variations in intracellular calcium can be a significant limitation on <math>Mg_i</math> determinations using this indicator. In order to overcome this shortcoming, we have devoted a significant effort toward the synthesis of indicators with improved selectivity for magnesium/calcium.</p>																	



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50106-02 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Collaborative Projects in Environmental Health Sciences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
Other:	Carol E. Parker	Chemist	LMB	NIEHS
Other:	Leesa Deterding/Steven	Chemist	LMB	NIEHS
	William Wilson	Research Chemist	LMIN	NIEHS
	Leo T. Burka	Research Chemist	LMB	NIEHS
	Frank Kari	Research Chemist	SBB	NIEHS
	Hideo Iwahashi	Visiting Scientist	LMB	NIEHS
	Ronald Mason	Research Chemist	LMB	NIEHS

## COOPERATING UNITS (if any)

Dr. D. Henke, Dept. Pulmonary Medicine, UNC Medical School, Chapel Hill, NC;  
 Dr. Michalopoulos, Dept. Pathology, Duke University Medical Center, Durham, NC.

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.15

## PROFESSIONAL:

0.9

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Collaborative projects in the environmental health sciences includes those projects in which the mass spectrometry work-group collaborates with other groups, both within and without the institute to solve problems of mutual interest. A major focus of these projects is the determination of xenobiotics in the environment, in living systems and the determination of their metabolic products. Their importance lies in the fact that the adverse interactions between compounds in the environment and their metabolite with living systems underlie our health concerns. These projects typically involve on-line separation and identification of complex mixtures and often involve use of all instrumental techniques available in the MS lab including thermospray LC/MS (TSP/LC/MS), FAB/MS and FAB/MS/MS (including the use of continuous flow techniques) and GC/MS.

A typical project is the identification of the metabolites of H.C. Blue No. 1 and H.C. Blue No. 2. H.C. Blue No. 1 is a known carcinogen which differs only slightly from the non-carcinogenic H.C. Blue No. 2. The metabolic profiles in mice of these two compounds differ significantly. H.C. Blue 2 was observed to yield one major metabolic product. Using GC/MS, HR/MS and TSP/MS technique, this product has been identified as a side chain oxidation product. In contrast, the carcinogenic H.C. Blue 1 undergoes dealkylation to the free amine, from which a variety of metabolites arise. The free aromatic amines are known carcinogens. Therefore, the difference in carcinogenic behavior can be explained on the basis of their different metabolisms.

Other projects, which are included in this heading, include analysis of airway epithelium prostaglandins, the determination of bradykinin in bovine milk, the identification of liver cell growth factor, and the identification of the products of free radicals with spin traps.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50107-02 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Nanoliter Capillary LC/MS Techniques

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Arthur Moseley	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	John Perkins	Visiting Fellow	LMB	NIEHS

## COOPERATING UNITS (if any)

Professor J. Jorgenson, Department of Chemistry, UNC, Chapel Hill, NC; Dr. D. Kassel, MIT, Massachusetts, General Hospital; Dr. D. Hunt, University of Virginia.

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.85

## PROFESSIONAL:

1.45

## OTHER:

1.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

A perennial problem in the mass spectrometric analysis of both biological and environmental samples is that the absolute level of analyte is extremely low. One approach to this problem is to develop low volume-high flux delivery systems for the mass spectrometer. We have undertaken the development of interfaces for nanoliter capillary systems and MS. These capillary systems offer the same advantages over wider-bore LC systems that capillary GC offers over packed-column GC, a high flux of analyte into the MS but with a significantly lower total analyte level necessary. Current developments are in the areas of continuous flow FAB (CF-FAB), and capillary zone electrophoresis (CZE). In the area of CF-FAB we have developed packed (50-75  $\mu$  id) columns for use in the analysis of peptide and protein digests. The greater loading capacity of these columns provides a higher dynamic range than do OTLC columns and is, thus, more suitable for complex mixtures and low level analyses. These column are also very compatible with MS/MS data acquisition. In the area of CZE we have successfully interface CZE with both CF-FAB and electrospray ionization. Both techniques have been found suitable for the determination of peptides and protein digests in the femtomolar concentration range. In conjunction with ESI and mass spectral data, proteins with MR  $\geq$  30,000 can be separated and their mass spectra obtained.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50108-02 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Development of Tandem Mass Spectrometry for Structure Elucidation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Kenneth Tomer Research Chemist LMB NIEHS

Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Steven McGown	Chemist	LMB	NIEHS
	Arthur Moseley	Chemist	LMB	NIEHS
	Leo Burka	Research Chemist	STB	NIEHS
	Thomas Eling	Research Chemist	LMB	NIEHS

## COOPERATING UNITS (if any)

Professor A. Spatola, University Louisville, NY; Drs. M.L. Gross and R.L. Cerny, University Nebraska; Dr. P. Thibault, Atlantic Research Laboratory, National Research Council, Canada

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.9

## PROFESSIONAL:

.55

## OTHER:

0.35

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major program in the mass spectrometry laboratory at NIEHS is the application of tandem mass spectrometric techniques (MS/MS) to the structure elucidation of compounds of interest in the environmental health sciences. The structure determination of these compounds is basic to understanding the interactions of compounds within the body, especially those due to altered metabolism and those arising through the interactions of xenobiotics and biomolecules. These techniques are important because samples of interest are often complex mixtures and because the ionization techniques applicable to these samples often provide little or no structural information.

Our approach to the development of MS/MS techniques is twofold; structure elucidation and increasing the sensitivity of the technique. Current projects in the area of structure determination include: 1) glutathione, cysteine and N-acetylcysteine conjugates of xenobiotics, including identification of conjugates excreted from challenged animals; 2) determination of the location of substituents and double bonds in compounds within the arachidonic acid cascade under negative ion FAB/MS/MS conditions; 3) development of hybrid MS/MS techniques selected reaction monitoring the analysis of polychlorinated aromatic compounds such as dibenzofurans and dibenzodioxins; and 4) comparison of the utility of hybrid MS/MS vs. high energy MS/MS. A major effort in increasing MS/MS sensitivity has been in the combination of high flux/low level introductory systems such as OTLC and CZE. We have successfully lowered the MS/MS acquisition levels several orders of magnitude for a number of analyte types including peptides, protein digests, nucleotides, carcinogen-modified nucleosides, phospholipids and carbohydrates.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50109-02 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peroxyl Free Radical Formation by Chloroperoxidase and Lipoxygenase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald P. Mason Research Chemist LMB NIEHS

Other: Walee Chamulitrat Visiting Fellow LMB NIEHS  
Thomas Eling Research Chemist LMB NIEHS  
Michael Hughes Research Chemist LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Electron Spin Resonance

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.2

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The decomposition of organic hydroperoxides as catalyzed by chloroperoxidase was investigated with electron spin resonance (ESR). Tertiary peroxy radicals were directly detected from incubations of *tert*-butyl hydroperoxide or cumene hydroperoxide with chloroperoxidase at pH 6.4. Peroxyl, alkoxyl, and carbon-centered free radicals from tertiary hydroperoxide/chloroperoxidase systems were successfully trapped by the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide, whereas alkoxyl radicals were not detected in the ethyl hydroperoxide/chloroperoxidase system. The classical peroxidase mechanism is proposed to describe the formation of peroxy radicals. In the case of tertiary peroxy radicals, their subsequent self-reactions result in the formation of alkoxyl free radicals and molecular oxygen. In the case of the primary ethyl peroxy radicals, decay through the Russell pathway forms molecular oxygen. Evidence for the production of singlet molecular oxygen was found.

The lipid peroxy radicals from the peroxidation of polyunsaturated fatty acids by soybean lipoxygenase were directly detected by the method of rapid-mixing, continuous flow ESR. When air-saturated, pH 9.0 borate buffer containing linoleic acid or arachidonic acid was mixed with lipoxygenase, fatty acid-derived peroxy free radicals were readily detected with a characteristic *g*-value of 2.014. Fatty acids without at least two double bonds, e.g., steric acid and oleic acid, did not give the corresponding peroxy free radicals, suggesting that the formation of a bisallylic carbon-centered radical preceded that of peroxy radical. The doublet feature of the arachidonate peroxy spectrum was proven (by selective deuteration) to be a hyperfine coupling due to a  $\gamma$ -hydrogen, which originated as a vinylic hydrogen of arachidonate.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 50110-02 LMB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>NMR Studies of Cellular Metabolism</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <b>PI: Robert E. London Research Physicist LMB NIEHS</b>  <b>Other: Xiaoming Wan Visiting Fellow LMB NIEHS</b> <b>Bruce Berkowitz Senior Staff Fellow LMB NIEHS</b> <b>Michael Perlman Senior Staff Fellow LMB NIEHS</b>		
COOPERATING UNITS (if any) <b>Professor Joseph J. Blum, Chairman, Division of Physiology, Department of Cell Biology, Duke University Medical Center, Durham, NC</b>		
LAB/BRANCH <b>Laboratory of Molecular Biophysics</b>		
SECTION <b>Nuclear Magnetic Resonance Group</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS: <b>2.0</b>	PROFESSIONAL: <b>1.3</b>	OTHER: <b>0.7</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>           This program is aimed at the development and application of <i>in vivo</i> NMR spectroscopic methods for studying metabolism and its perturbation by chemical toxins. A principal focus of these studies has been the development of NMR active, intracellular indicator molecules to allow determination of metabolic parameters of interest in intact cells. Research has focused on the use of fluorinated indicators as a consequence of the inherent sensitivity of fluorine for NMR detection and the essential absence of background fluorine resonances from untreated cells. We have previously noted that in NMR studies of suspensions of human erythrocytes to which fluorine-containing molecules have been added, there is generally a significant chemical shift difference between intra- and extracellular indicators. Use was made of this shift difference to study the distribution of various molecules between intra- and extracellular spaces. We have subsequently initiated studies of the transport of fluorinated nucleosides, making use of magnetization transfer methods which allow the study of rapidly transported molecules under steady state conditions. Erythrocytes have frequently served as models for transport studies since there is generally a minimum of interference due to other metabolic transformations which can complicate the analysis. In addition, we have recently evaluated the use of perfluorotributylamine (FTBA) as an indicator of partial oxygen pressure <i>in vivo</i>. We are evaluating this technique in the gas-compressed vitrectomized rabbit eye; the high precision of this approach has allowed the rate of oxygen flow into the vitreous to be studied in real time. It was found that the vitreous substitute (FTBA) acts as an oxygen reservoir with a <i>pseudo</i>-first order half life of oxygen elimination from FTBA in the intact eye of roughly 60 minutes. NMR studies of the metabolism of polyphosphates by the protozoan <i>Leishmania</i> major, responsible for causing the disease <i>Leishmania</i>, have also continued.         </p>		



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <div style="text-align: center; font-weight: bold;">Z01 ES 50111-02 LMB</div>
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>NMR Studies of Biomolecular Structure, Function, and Dynamics</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
<b>PI:</b> Robert E. London	Research Physicist	LMB NIEHS
<b>Other:</b> Donald G. Davis Michael E. Perlman	Expert Senior Staff Fellow	LMB NIEHS LMB NIEHS
COOPERATING UNITS (if any) Dr. Morrow Thompson, DTRT; Dr. Robert Handschumacher, Department of Pharmacology, Yale University School of Medicine, New Haven, CT; Dr. John M. Stewart, Department of Biochemistry, University of Colorado Health Science Center, Denver, CO.		
LAB/BRANCH Laboratory of Molecular Biophysics		
SECTION Nuclear Magnetic Resonance Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 2.3	PROFESSIONAL: 1.6	OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have continued our efforts to modify and improve NMR methodologies for the assignment of resonances and the analysis of molecular structure and dynamics, and to apply these approaches to problems related to environmental health. One of the central problems inherent in the analysis of complex molecules using NMR is the complexity of the spectra resulting from the large number of overlapping resonances. Several new strategies for obtaining edited 2-dimensional NMR spectra which overcame the limitations resulting from the large number of resonances have been evaluated over the past year. In collaboration with Dr. Morrow Thompson of DTRT, some of these methods have been applied to the analysis of bile acid adducts obtained subsequent to the treatment of rats with a-naphthyl isothiocyanate (ANIT) which causes bile duct necrosis. Two new structural NMR efforts, both related to AIDS research, were also initiated during the past year. The first is a collaborative effort with Dr. Robert Handschumacher on the enzyme cyclophilin, the apparent target of the immunosuppressive drug cyclosporin A. It has recently been demonstrated that cyclophilin catalyzes the <i>cis/trans</i> isomerization of peptidyl-proline bonds; however, the physiologically important targets of this enzyme have yet to be identified. It was proposed that the biologically active peptide bradykinin might be a substrate for this enzyme, and fluorinated analogs of bradykinin which can be monitored with fluorine-19 NMR were prepared by Dr. John Stewart of the University of Colorado. It was been determined that the <i>cis/trans</i> isomerization rate constants of both bradykinin and its [Gly <sup>6</sup> ]-bradykinin analog are significantly increased in the presence of the enzyme. A second structural study initiated during the past year is aimed at determining the conformation of inhibitors of the enzyme purine nucleoside phosphorylase (PNPase), which is important in the catabolism of nucleoside drugs used to treat a variety of illnesses, as well as in the synthesis of the these drugs.		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50112-02 LMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Magnetic Resonance Imaging Studies of Heavy Metal Distribution

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert E. London	Research Physicist	LMB	NIEHS
Other:	Xiaoming Wan	Visiting Fellow	LMB	NIEHS
	Bruce Berkowitz	Senior Staff Fellow	LMB	NIEHS
	Donald G. Davis	Expert	LMB	NIEHS

COOPERATING UNITS (if any)

Dr. James R. Brainard, INC-4, Los Alamos National Laboratory, Los Alamos, NM.

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Nuclear Magnetic Resonance Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.7

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has recently become possible to obtain spatially resolved "images" of the nuclear spins of biological and chemical materials. Magnetic resonance imaging (MRI) is rapidly evolving into an important tool for the clinical diagnosis of a wide range of human pathologies. Such imaging studies have been almost exclusively limited to the detection of protons, which provide images of the abundant protonated molecules in biological tissues: fat and water. Since image intensity is dependent on the density of protons in a given sample volume, as well as on the nuclear relaxation properties of these protons, it becomes possible to study the distribution of species which can alter these nuclear relaxation parameters. This dependence is the basis for the use of so called "contrast agents", most typically chelated paramagnetic ions, which enhance the contrast of regions into which they are transported as a result of their effects on relaxation parameters. In general, the use of such agents may be associated with additional toxicity. We have carried out studies of the distribution of several of these compounds, including a relatively new, experimental agent: GdHam, which is somewhat unusual in that it carries a net positive charge. In contrast to previous studies in which the agents are introduced via intraperitoneal or intravenous injection, these agents were administered to rats intracerebrally via the lateral ventricle. This route of administration allowed us to study the dynamics of the cerebrospinal fluid. One interesting result of this study is that the distribution of the agents tested appears to depend on the net charge, with positively charged molecules such as GdHam tending to associate with the ventricular surfaces. In addition, intracerebral administration of GdHam minimized the respiratory distress which we have noted to be associated with intravenous intraperitoneal administration of this agent. MRI studies using intracerebrally administered nitroxides as contrast agents have also been carried out during the past year.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 50113-02 LMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Free Radical Metabolite of Acetaminophen

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ramakrishna Rao	Visiting Associate	LMB	NIEHS
Other:	Ronald P. Mason	Research Chemist	LMB	NIEHS
	Sandra Jordan	Biologist	LMB	NIEHS
	David Duling	Programmer/Analyst	LMB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biophysics

SECTION

Molecular Biophysics

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been discontinued.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50114-02 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Xenobiotic Metabolism in Lower Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Phillip W. Albro Research Chemist

LMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Metabolism

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.3

## PROFESSIONAL:

0.7

## OTHER:

1.6

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has two objectives: (1) To explore the metabolic capabilities of invertebrate species, with emphasis on the ability to metabolize common environmental pollutants. Initially we are studying compounds whose metabolism is well understood in mammals, in order to make comparisons. (2) To investigate the possibility that some types of metabolism studies, especially those which must be performed *in vivo*, can be effectively accomplished in species having less developed nervous systems (and are thus presumably less subject to pain and distress) than the more commonly used rodent species. We are presently studying *Lumbricus terrestris*, the common earthworm ("night crawler") because it has been relatively neglected in studies of metabolic capabilities, and because it is typically exposed to environmental pollutants in landfills. We have observed that while *L. terrestris* appears to metabolize p,p-DDT, adipate diester, and polychlorinated biphenyls in a manner qualitatively similar to higher animals, the metabolism of phthalate diesters and of phthalic acid itself by earthworms differs considerably from the pattern seen in both higher and lower species. In particular, *L. terrestris* seems to lack the ability to oxidize the ester side chain of ethylhexyl phthalate, and has the ability (unique among animals tested) to oxidize free phthalic acid completely to carbon dioxide. The latter pathway is currently under investigation. We have also characterized the lipids of *L. terrestris*, and found them to be extremely complex. The fatty acid compositions of lipid classes differ considerably from what has been reported for this species, due to the increased resolving power of modern capillary column chromatography. Earthworms contain a complex mixture of neutral sterols, which has been resolved and fully characterized for the first time. Glycolipids are major components of the extractable lipids, and have never previously been characterized.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 ES 50115-02 LMB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>A Computerized Spin Trapping Data Base</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
<b>PI:</b>	<b>Colin F. Chignell</b> <b>Anson S.W. Li</b>	<div style="display: flex; justify-content: space-between;"> <div> <b>Chief</b>  <b>Staff Specialist</b> </div> <div style="text-align: right;"> <b>LMB NIEHS</b>  <b>LMB NIEHS</b> </div> </div>
COOPERATING UNITS (if any)  <b>Dr. Garry R. Buettner, University of Iowa</b>		
LAB/BRANCH <b>Laboratory of Molecular Biophysics</b>		
SECTION <b>Molecular Biophysics</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS: <b>0.9</b>	PROFESSIONAL: <b>0.4</b>	OTHER: <b>0.5</b>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>Spin trapping is a powerful and convenient technique for the study of free radical reactions. The breadth of applications ranges from clinical studies to high-energy physics. Over 1500 references to the technique have accumulated in Chemical Abstracts. STDBII, a spin trapping database, has been implemented on IBM PC/AT and Macintosh personal computers. The package operates with no "add-ons." The program is powerful yet user-friendly; the command structure is similar to the familiar 1-2-3 light-bar menu; search strategy employs the method of Query-by-Example (QBE); logical combination of any fields is accomplished by using AND, OR, NOR, and EXCEPT. Presently, STDBII (4.0) contains files for 5,5-dimethylpyrroline-N-oxide (DMPO), alpha-phenyl-N-tert-butyl nitron (PBN), 2-methyl-2-nitrosopropane (MNP), alpha-(4-pyridyl-1-oxide)-N-tert-butyl nitron (POBN), nitrosodurene (ND) and 3,5-dibromo-nitrosobenzene sulfonate (DBNBS). Data for other less popular traps are included in a catch-all file. Our goal is to incorporate all published work that relates to spin trapping. Presently, the database files have more than 1500 references with over 5500 parameter entries. The STDBII files contain information on: 1) spin trap used; 2) radical trapped; 3) hyperfine splittings reported; 4) solvent; 5) g-value, if reported; 6) a terse summary on how the radical was produced and observed; 7) full bibliographic data; and 8) retraction on anything by the author. STDBII helps researchers: 1) in identification of spin adducts from the sometimes unique hyperfine splitting parameters; 2) as a key to the spin trapping literature 3) as a vehicle to correct published errors. STDBII is now available to researchers both inside and outside NIEHS. The package includes a user manual that lists all of the compiled information on spin trapping. Scientists who do not presently have access to an IBM/PC can still benefit from STDBII because all of the database entries are printed in the STDBII User Manual.</p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 50116-01 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Microdialysis/Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kenneth B. Tomer	Research Chemist	LMB	NIEHS
Other:	Leesa Deterding	Chemist	LMB	NIEHS
	Arthur Moseley	Chemist	LMB	NIEHS
	Leo T. Burka	Research Chemist	STB	NIEHS
	Kelly Washburn	Guest Researcher	STB	NIEHS
	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
	Wanqin Zhang	Visiting Fellow	LMIN	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Mass Spectrometry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.4

## PROFESSIONAL:

0.1

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

We have recently coupled microdialysis with coaxial continuous flow FAB mass spectrometry. This technique allows for the possibility of determination in real time of many biochemicals in the body. Microdialysis is a relatively new sampling technique designed for the in vivo analysis of chemical substances. The microdialysis probe is surgically implanted into the tissue or area of interest in the animal. This in vivo technique is advantageous since there is minimal damage to the sampling site and it does not alter the fluid balance.

The present investigation involves the possibility of determining the half-life of a *tris* organophosphate, *tris* (2-chloroethyl) phosphate (TRCP), in rats. Several of these *tris* organophosphates have been widely used in plastics and synthetic fibers as flame retardants and are suspected carcinogens. In our initial experiments, a decline in the amount of TRCP in the blood stream of anesthetized rats was observed over a three hour time period after dosing the animal. We have just completed a surgical protocol in order to perform the same experiments in awake, freely-moving animals. This will allow a more realistic determination of the physiological levels in the animal. In the future, we will be performing microdialysis in the brain of rats in an effort to determine the levels of endogenous neuropeptides and neurotransmitters in real time.







## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80008-15 LMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Prostaglandins, Hydroxy-Fatty Acids and Leukotrienes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas E. Eling	Research Chemist	LMB	NIEHS
Other:	Wayne Glasgow	Staff Fellow	LMB	NIEHS
	Julie Angerman-Stewart	Biologist	LMB	NIEHS
	Janet Capps	Biologist	LMB	NIEHS
	Carl Barrett	Research Chemist	LMB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Biophysics

## SECTION

Eicosanoid Biochemistry

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.8

## PROFESSIONAL:

2.6

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations are concerned with the oxidation of arachidonic acid to prostaglandins (PG), leukotrienes and hydroxy-fatty acids and the relationship of this metabolism to the regulation or modulation of biological processes. We have investigated the mechanism responsible for the inhibition of PHS by peroxidase for inhibition to occur. We have also studied the role of arachidonic acid metabolism in the response of cells to growth factors. For BALBc cells, PGs are required for EGF but not PDGF stimulated DNA synthesis. The addition of EGF stimulates PG formation and the expression of c-myc occurs after PG formation. Inhibition of PG formation inhibits the expression of c-myc while the addition of PG restores the c-myc. Thus in these cells c-myc expression appears to be modulated by PGs. In contrast PGs are potent inhibitors of EGF-stimulated DNA synthesis in Syrian hamster embryo (SHE) cells. In response to EGF both the BALBc cells and SHE cells metabolize linoleic acid to 9/13-hydroxyoctadecadienoic acid (9/13-HODD), which when added to these cells enhances DNA synthesis. Inhibition of the 15-lipoxygenase, that catalyzes this oxidation, inhibits DNA synthesis. The addition of 9/13-S-HODD significantly stimulated EGF dependent mitogenesis at concentration as low as  $10^{-10}$ M. Moreover, EGF is required for the lipoxygenase to oxidize linoleic acid to 9/13-HODD which suggest that EGF induces or activates the lipoxygenase. The data indicate that EGF-stimulated DNA synthesis requires the linoleic acid metabolites and that growth factors either activate or induce the synthesis of the 15-lipoxygenase. Studies are currently underway to further investigate these problems. These findings suggest a possible important role for arachidonic and linoleic acid metabolism in regulating cell growth.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 ES 80035-14 LMB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Cooxidation of Xenobiotics by the Prostaglandin</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Thomas E. Eling	Research Chemist LMB NIEHS
Other:	Ronald P. Mason	Research Chemist LMB NIEHS
	David P. Thompson	Staff Fellow LMB NIEHS
	Bill J. Smith	IRT LMB NIEHS
	Michael Hughes	Staff Fellow LMB NIEHS
	Richard Philpot	Research Chemist LMB NIEHS
	Yoland Van der Zee	Visiting Fellow LMB NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Molecular Biophysics		
SECTION Eicosanoid Biochemistry		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 4.1	PROFESSIONAL: 3.1	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             The long range goal of this project is to study the oxidation of chemicals to toxic or carcinogenic metabolites by prostaglandin H synthase (PHS) and to demonstrate the importance of this enzyme system in chemical-induced toxicity or carcinogenesis. We have shown that aromatic amine carcinogens, are metabolized to mutagens by PHS. PHS dependent oxidation occurred by a free radical mechanism and resulted in the formation of DNA adducts which can be used as <i>in vivo</i> markers for PHS-dependent oxidation. We have further studied the formation of amine mutagens by PHS using bacterial tester systems having different levels of acetylase activity. Our data indicate that acetylase plays an important role in the formation of free radical mutagens from aromatic amines, including bladder carcinogen such as benzidine derivatives. We are currently investigating the differences between PHS and HRP as related to the formation of mutagens by these peroxidases and the formation of oxygenated metabolites of aromatic amines by PHS. We further studied peroxidase catalyzed GSH conjugate formation and showed that this reaction occurs with a number of chemicals that contain a conjugated double bond adjacent to an aromatic ring. The reaction appears to be a general mechanism for conjugate formation. We have also shown that P-450 metabolites of BP will enhance this reaction which serves as a mechanism for detoxication of carcinogens. We have investigated the dealkylation of a series of N-substituted aromatic amines by peroxidases. The mechanism by the removal and oxidation of the alkyl group depends on the chemical nature of the group. Our data suggest that PHS is a versatile enzyme system that can catalyze a variety of reactions which are important in the conversion of chemicals to carcinogenic metabolites in extra hepatic tissue.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 23000-03 LMC

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Events in Multistep Carcinogenesis of Mice

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.W. Wiseman Senior Staff Fellow LMC NIEHS

Others: C.J. Cochran Biologist LMC NIEHS

## COOPERATING UNITS (if any)

DIR/LMC, NIEHS (J.C. Barrett) DTRT/CGTB, NIEHS (W.D. Caspary)  
University of North Carolina (V. Bautch)  
DIR/LRDT, NIEHS (E.M. Eddy and E.F. Goulding)

## LAB/BRANCH

Laboratory of Molecular Carcinogenesis

## SECTION

Chemical Carcinogenesis

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.75

## PROFESSIONAL:

0.75

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to identify genetic alterations in tumor suppressor genes and proto-oncogenes that play a role in mouse carcinogenesis. Allelic losses of specific chromosomal regions, detected by restriction fragment length polymorphism (RFLP) analyses, suggest that tumor suppressor gene inactivation occurs in most common human cancers. We have extended RFLP analyses to F1 mouse tumor DNA in order to map potential tumor suppressor genes in mice. DNA from a panel of butadiene-induced lung adenocarcinomas, lymphomas, and hepatomas of B6C3F1 mice (NTP bioassay) have been examined for allelic losses. RFLP analyses revealed that one copy of chromosome 4 was deleted in five of eight lung tumors as well as one of ten lymphomas; one allele of the retinoblastoma gene was also deleted in a lung tumor. Lung tumor DNAs were examined by PCR and direct sequencing for point mutations in the P53 tumor suppressor gene, which is frequently mutated in a wide variety of human tumors. A G→T transition in a P53 mutation hotspot was observed in one tumor. RFLP analysis and a novel PCR assay for a CA repeat near the P53 gene revealed allelic losses in DNA of another lung tumor and three lymphomas. These tumors are likely to contain mutant P53 genes which we will characterize by single-strand conformation polymorphism analyses and direct sequencing. In order to generate additional tumors for RFLP studies, we have constructed a series of 9 transgenic mouse lines containing mutated mouse P53 gene constructs in collaboration with M. Eddy and G. Goulding (LRDT). These lines are currently being characterized for transgene expression and tumor formation (lung adenocarcinomas, osteosarcomas, and lymphomas). In addition we have obtained transgenic lines that develop mammary carcinomas (MMTV-ras, MMTV-neu, and MMTV-myc), osteosarcomas (polyoma early region) and lung carcinomas (Abl-ras) from outside investigators for analysis of allelic losses and P53 mutations.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 23001-01 LMC

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Human Gynecologic Pathology

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.A. Boyd Senior Staff Fellow LMC NIEHS

Others: J.I. Risinger Biologist LMC NIEHS

## COOPERATING UNITS (if any)

DIR/LMC, NIEHS (J.C. Barrett) Duke University Medical  
University of N.C. (J.M. Schildkraut, G.A. Dent) Center (D. Walmer)  
Montreal General Hospital (M.-S. Tsao)

## LAB/BRANCH

Laboratory of Molecular Carcinogenesis

## SECTION

Gene Expression

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.50

## PROFESSIONAL:

0.25

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project include the analysis of alterations in oncogenes and tumor suppressor genes in human endometrial carcinoma. PCR amplification, Southern blotting, and northern blotting procedures have been successfully employed to evaluate oncogene activation (particularly of the K-ras gene) and tumor suppressor gene inactivation (particularly of the DCC gene) in 12 human endometrial carcinoma cell lines. These studies will be extended to primary human tumor specimens, both fresh and archival paraffin-embedded specimens, as well as to fresh tissue from several pre-malignant endometrial conditions. Similar strategies will be employed to evaluate potential molecular genetic alterations in endometriosis, especially as they may relate to those changes found in endometrial carcinoma. Endometriosis is a non-malignant condition of aberrant endometrial tissue growth, affecting 10-15% of the premenopausal U.S. population. In addition, collaborative studies are underway, the goals of which are to map tumor suppressor gene alterations that are common to tumors arising in a familial breast-ovarian cancer syndrome. PCR amplification and Southern blotting procedures will also be employed to analyze loss of heterozygosity at restriction fragment length polymorphic loci in familial breast and ovarian carcinoma DNA. The DNA from fixed and paraffin-embedded tumors was successfully extracted and analyzed as a prelude to these studies.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 25001-13 LMC

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Mutagenesis in Carcinogenesis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.C. Barrett Research Chemist LMC NIEHS

Others: P. Lamb Biologist LMC NIEHS

## COOPERATING UNITS (if any)

Laboratory of Reproductive and Developmental Toxicology, DIR (Dr. J. McLachlan)  
Mt. Sinai Hospital (Dr. N. Suzuki)  
Nippon Dental University, Tokyo (Dr. T. Tsutsui)

## LAB/BRANCH

Laboratory of Molecular Carcinogenesis

## SECTION

Cellular Carcinogenesis

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.0

## OTHER:

.05

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Most chemical carcinogens induce DNA damage and are mutagenic at specific genetic loci; however, certain carcinogens (including the human carcinogens diethylstilbestrol (DES), asbestos, arsenicals and benzene) usually do not induce gene mutations. We have examined the ability of these chemicals to induce morphological transformation, gene mutations and chromosome mutations in Syrian hamster embryo (SHE) cells in culture. We have previously proposed that the mechanism of action of DES is related to its ability to induce numerical chromosome changes, i.e., aneuploidy. Currently, DES-induced aneuploidy is being examined in the newborn mouse genital tract to test whether these changes occur in vivo in the target tissue. The mechanism of another important human carcinogen, asbestos, was also examined. We have proposed that asbestos induces cell transformation due to its ability to induce chromosomal changes. We have identified a possibly novel transforming oncogene in human mesotheliomas, and currently we are cloning this gene. Sodium arsenite and sodium arsenate are inactive as gene mutagens but are potent inducers of cell transformation, chromosome aberrations and gene amplification. Benzene induces cell transformation but is a weak gene mutagen. This chemical is a very effective inducer of aneuploidy in this system. These results further support our hypothesis that cell transformation involves a chromosomal mutation and suggest an important role for carcinogen-induced aneuploidy in carcinogenesis. Di(2-ethylhexyl)phthalate (DEHP), a commonly used plasticizer, induces peroxisome proliferation in liver cells and hepatocellular carcinomas in rodents. We have shown that DEHP induces morphological transformation, chromosome aberrations, and peroxisome proliferations of cultured Syrian hamster embryo (SHE) cells. The transformation frequency and chromosomal aberrations by DEHP was enhanced in the presence of rat liver post-mitochondrial supernatant. The results suggest a possible involvement of genetic damage by DEHP metabolites in the induction of transformation of SHE cells. No clear relationship between induction of peroxisome proliferation and cell transformation was observed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 25031-04 LMC

## PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Tumor Suppressor Genes and Oncogenes in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.C. Barrett	Research Chemist	LMC	NIEHS
Others:	J. Hosoi	Visiting Fellow	LMC	NIEHS
	J. Stowers	IRTA Fellow	LMC	NIEHS
	H. Satoh	Visiting Fellow	LMC	NIEHS
	H. Yamada	Visiting Associate	LMC	NIEHS
	C. Afshari	preIRTA Fellow	LMC	NIEHS
	R. Whitehead	P Appointment	LMC	NIEHS

## COOPERATING UNITS (if any)

Gene Expression Group, LMC  
 Chemical Carcinogenesis, LMC  
 Tottori University (Dr. M. Oshimura)

## LAB/BRANCH

Laboratory of Molecular Carcinogenesis

## SECTION

Cellular Carcinogenesis

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

6.5

## PROFESSIONAL:

5.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☒ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cancer development in humans and animals is a multistep process involving at least two classes of genes, proto-oncogenes and tumor suppressor genes. We have shown that neoplastic transformation of Syrian hamster embryo cells (SHE) in culture is a multistep process involving both activation of proto-oncogenes and inactivation of a tumor suppressor gene. The loss or inactivation of tumor suppressor genes is an essential step in the multistep neoplastic transformation of SHE cells. Non-tumorigenic variants have been isolated that have lost (sup<sup>-</sup>) or retained (sup<sup>+</sup>) the ability to suppress tumorigenicity of tumor cells in cell hybrids. Fusions of sup<sup>+</sup> or sup<sup>-</sup> variants with different tumor cells show different patterns of suppression indicating that a family of tumor suppressor genes exists in these fibroblast cells. Currently, several strategies to clone tumor suppressor genes are in progress. cDNA libraries of sup<sup>+</sup> hamster cells have been screened with RNA from sup<sup>+</sup> or sup<sup>-</sup> cells and differentially expressed cDNAs have been cloned. Two-dimensional gel analyses of proteins showed that a reduction in the expression of tropomyosin I correlates with the loss of the tumor suppressor function. A cellular phenotype associated with the loss of tumor suppressor gene function has also been found. Sup<sup>+</sup> cells suspended in agar respond reversibly to transforming and normal growth factors by forming colonies in agar whereas sup<sup>+</sup> cells fail to grow. Tumor suppressor genes can be mapped to specific chromosomes by introduction of normal chromosomes into tumor cells by microcell fusion. We have shown that normal human chromosome 11 suppresses cervical carcinoma cells, lung adenocarcinoma cells, rhabdomyosarcoma cells, and Wilms' tumor cells, whereas chromosome 3 suppresses renal carcinoma and lung adenocarcinoma cells. An uterine endometrial cancer cell was suppressed by three different chromosomes (Nos. 1, 6, and 9). In addition to the tumor suppressor genes described above that are expressed in some immortal cell lines, tumorigenicity also may be limited by cellular senescence. Our results indicate that a gene(s), possibly involved in the senescence phenotype, can be mapped to human chromosome 1.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 60147-07 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of SOS-Mutagenesis in Escherichia coli

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. M. Schaaper Visiting Scientist LMG NIEHS

Others: I. Fijalkowska Visiting Fellow LMG NIEHS  
R. L. Dunn Biologist LMG NIEHS  
R. Cornacchio Stay-In-School Employee LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

2.25

PROFESSIONAL

1.5

OTHER

0.75

CHECK APPROPRIATE BOXES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The SOS system of Escherichia coli plays a central role in mutagenesis in this organism. The system is not normally present in the cell but becomes induced upon blockage of DNA replication by DNA damage. Its induction entails the expression of a large number of gene products, several of which are postulated to interact with the process of DNA replication, rendering it error prone and producing mutations on both damaged and undamaged DNA. The evidence for the existence of these components rests largely on genetic experiments. However, the elucidation of the nature of these components and their mechanisms of action requires a more direct biochemical approach. We have designed an in vitro DNA replication system in which the existence of the error-prone replication components may be tested. The system uses the conversion of single-stranded bacteriophage M13 DNA to its double-stranded form (ss → RF conversion) by cell-free extracts derived from either normal or SOS-induced cells. After replication, the product DNA is transfected to produce intact bacteriophage. The accuracy of the in vitro replication step is determined from the frequency of mutant phage before and after replication. Since insights into SOS-modified DNA replication requires knowledge of the factors involved in maintaining normal accuracy, the latter is investigated as well, using, amongst others, E. coli mutator strains with known (or presumed) DNA replication defects. We have found DNA replication in E. coli extracts to be extremely accurate, with error rates approaching (or identical to) estimated in vivo rates. Extracts of two different E. coli mutator strains, mutD and mutT, display enhanced error rates in this assay. In case of mutT, this observation was exploited to uncover the role of the mutT gene product, namely to specifically prevent the otherwise frequent misinsertion of dGTP opposite template adenines. The system is currently used to investigate error rates during DNA synthesis by extracts from cells induced for the SOS response.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 61022-09 LMG</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>The Population Genetics of Transposable Elements</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
<b>PI:</b>	<b>C. H. Langley</b>	<b>Research Geneticist LMG, NIEHS</b>
<b>Others:</b>	<b>E. A. Goode-Montgomery</b> <b>G. M. Simmons</b> <b>W. H. Stephan</b> <b>B. H. Judd</b> <b>S. M. Huang</b>	<b>Geneticist LMG, NIEHS</b> <b>Staff Fellow LMG, NIEHS</b> <b>Special Volunteer LMG, NIEHS</b> <b>Research Geneticist LMG, NIEHS</b> <b>Geneticist LMG, NIEHS</b>
COOPERATING UNITS (if any) <b>Dr. N. Kaplan and R. Hudson, Biometry and Risk Assessment Program</b> <b>Dr. Brian Charlesworth, Department of Biology, University of Chicago</b>		
LAB/BRANCH <b>Laboratory of Molecular Genetics</b>		
SECTION <b>Eukaryotic Gene Structure and Function Section</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS: <div style="text-align: center;"><b>2.0</b></div>	PROFESSIONAL: <div style="text-align: center;"><b>1.5</b></div>	OTHER <div style="text-align: center;"><b>.5</b></div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects  <input type="checkbox"/> (a1) Minors  <input type="checkbox"/> (a2) Interviews         </div> <div> <input type="checkbox"/> (b) Human tissues         </div> <div> <input checked="" type="checkbox"/> (c) Neither         </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>The main goal of this project is to study the population biology of transposable genetic elements (parasites of the genome) using <i>Drosophila</i> as a model system in conjunction with quantitative theoretical analysis. During this period, the research has focused on two topics: 1) What is the primary mechanism containing the numbers of transposable elements? and 2) Is the evolutionary diversity observed between copies of elements at the DNA sequence level consistent with quantitative models of the dynamics of the elements in natural populations? The cloning and DNA sequencing of copies of the transposable element <i>hobo</i> from sampled individuals, populations and species is ongoing. The genetic and molecular characteristics of spontaneous deletions arising from unequal crossing over is ongoing. The role of heterozygosity on the rate of unequal crossing over is under investigation.</p> <p>Following the departure of Drs. Langley, Simmons and Stephan, this project is continuing as a collaboration between Dr. Langley at the University of California, Davis and the Judd group at NIEHS (see Z01 ES 65037-06 LMG).</p>		





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61024-08 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Molecular Analysis of Suppressor-of-Sable Function in *Drosophila*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. A. Voelker	Research Geneticist	LMG, NIEHS
Others:	M. T. Eisenberg	IRTA	LMG, NIEHS
	J. F. Sterling	Biologist	LMG, NIEHS
	J. P. Graves	Biologist	LMG, NIEHS
	T. J. Maness	Biological Aid (SIS)	LMG, NIEHS
	S. Lingle	Biological Aid (SIS)	LMG, NIEHS
	S. R. Lamprides	Biological Aid (SIS)	LMG, NIEHS
	T. V. Garland	Biological Aid (SIS)	LMG, NIEHS
COOPERATING	W. K. Jarchow	Summer IRTA	LMG, NIEHS
	K. K. Wehrle	Summer IRTA	LMG, NIEHS

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Eukaryotic Gene Structure and Function Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

6.4

## PROFESSIONAL:

2.0

## OTHER:

4.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating the molecular mechanism of action of the *suppressor-of-sable* [*su(s)*] system of *Drosophila melanogaster*: recessive *su(s)* mutations suppress recessive mutations at the *vermillion* (*v*) locus that are caused by insertions of the mobile element 412 in 5' transcribed but untranslated sequences. Current evidence suggests that this suppression is effected by some mechanism that increases the amount of pseudo-wild-type mRNA that is produced by splicing the mobile element sequences from the primary transcript.

The cDNA contains an open reading frame that encodes a putative protein of 1322 amino acids, and cellular fractionation studies have shown that the protein is primarily located in the nucleus. This protein has one region of similarity to the RNA Recognition Motif (RRM) that is found in many proteins that are involved in RNA processing and a second region of similarity to the human, *Xenopus* and *Drosophila* 70K U1 binding proteins and to the *Drosophila suppressor of white-apricot* and *transformer* proteins, all of which are known to be RNA binding proteins. Portions of the cloned 25 kb of DNA have been reintroduced by P element mediated transformation and allow an identification of genetic function with messages produced by the region. A segment of DNA which is homologous with only the the *su(s)* message rescues both the primary phenotype of suppression and a secondary phenotype of cold-sensitive male sterility. Studies of genetic deletions indicate that the females lacking the entire *su(s)* protein are viable and fertile and that males lacking more than half of the protein are viable and fertile.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 61037-06 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of DNA Replication in Eucaryotes: Yeast as a Model System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Sugino	Visiting Scientist	LMG NIEHS
Others:	A. Morrison	Visiting Scientist	LMG NIEHS
	H. Araki	Visiting Associate	LMG NIEHS
	K. Kitada	Visiting Associate	LMG NIEHS
	L. Amin	IRTA Fellow	LMG NIEHS
	A. B. Clark	Biologist	LMG NIEHS
	L. Nurre	Biologist	LMG NIEHS
	A. Negishi	Summer IRTA Fellow	LMG NIEHS
	T. Sugino	Guest Worker	LMG NIEHS

COOPERATING UNITS (If any) Dr. L. H. Johnston, Group Leader, Lab. Cell Propagation, Nat. Inst. for Med. Res., London, England; Prof. T. Wang, Dept. of Pathology, Stanford Univ., Sch. of Med., Stanford, CA; Prof. R. A. Bambara, Dept. of Biochem., Univ. of Rochester, Rochester, NY

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

5.9

3.65

2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to establish an *in vivo* function of *Saccharomyces cerevisiae* DNA polymerase II (a homolog of mammalian DNA polymerase  $\epsilon$ ) which we have identified and purified last year, the gene has been identified, cloned by both antiserum against the purified DNA polymerase II and its partial amino acid sequence and its nucleotide sequence have been determined. Mutants having various deletions and disrupted gene have been generated. Using these mutants, it has been shown that DNA polymerase II is an essential enzyme for yeast chromosome replication and it is conceivable that DNA polymerase  $\epsilon$  could be an essential enzyme for DNA replication in mammalian cells. Based upon these results, we have proposed a new model to explain how eucaryotic chromosome is replicated by three DNA polymerases inside the cells. We also have cloned and sequences the genes encoding DNA polymerase II accessory proteins (subunits B, C, and D).

CDC7 gene is well known to be required for initiation of chromosome replication, but not an origin-specific binding protein. We have isolated and sequenced one gene which is able to suppress *cdc7* mutations. This gene is an already known cell-division-cycle gene, *DBF4*, which we have been studying independently from the above mentioned. From this, we have concluded that the *DBF4* protein interacts directly with the *CDC7* protein (associated with a protein kinase activity) to facilitate initiation of chromosome replication during S-phase in *S. cerevisiae*.

Another DNA replication protein that we have studied in this year is a new type of single-stranded DNA binding protein which consists of three different polypeptides. This protein complex is a homolog of human RF-A protein. This protein greatly stimulates DNA polymerase I reaction, but has little effect on either DNA polymerase II or III reaction. Most of the protein localizes in yeast nuclear matrix, suggesting that it plays a very important role for chromosome replication.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61039-06 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Mechanisms of DNA Recombination and Repair in the Yeast Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Sugino Visiting Scientist LMG NIEHS

Others: A. Morrison Visiting Scientist LMG NIEHS  
K. Kitada Visiting Associate LMG NIEHS  
A. B. Clark Biologist LMG NIEHS  
T. Sugino Guest Worker LMG NIEHS

## COOPERATING UNITS (if any)

Dr. C. C. Dykstra, Ass. Prof., Dept. of Pathology, Univ. of NC, Chapel Hill, NC;  
Dr. E. C. Friedberg, Prof., Dept. of Pathology, Stanford Univ., Sch. of Med.,  
Stanford, CA

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS.

1.5

## PROFESSIONAL

1.2

## OTHER

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

An ATP-independent DNA strand-transfer protein has been identified, purified and characterized from both mitotic and meiotic Saccharomyces cerevisiae cells. We have named them STP $\alpha$  and  $\beta$ , respectively. Using antiserum and the partial amino acid sequence information from the purified proteins, the respective genes have been cloned and sequenced. Although the STP $\alpha$  gene (DST1) is not required for mitotic growth, it is required for normal level of meiosis-specific homologous recombination. On the other hand, the STP $\beta$  gene (DST2) is required for meiosis and the gene disruption mutation of the gene exhibited reduced level of meiotic homologous recombination as well as lower level of mitotic homologous recombination. These data have proven that STP is one of homologous recombination components in yeast and an ATP-independent DNA strand transfer reactions catalyzed by STP are biologically important. Furthermore, we have shown that RAD3 protein, at least *in vitro*, interacts with STP proteins and stimulates DNA strand-transfer reactions by an ATP-independent manner.

In addition, antiserum against yeast RAD6, RAD18, and REV3 gene products have been raised to establish a protocol for their protein purification.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61041-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular genetic variation in natural populations

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Charles H. Langley	Research Geneticist	LMG, NIEHS
Others:	Naohiko Miyashita	Visiting Fellow	LMG, NIEHS
	Gail M. Simmons	Staff Fellow	LMG, NIEHS
	Wolfgang Stephan	Special Volunteer	LMG, NIEHS
	William Quattlebaum	Biologist	LMG, NIEHS
	Beatriz Boñi	Visiting Fellow	LMG, NIEHS

## COOPERATING UNITS (if any)

Dr. Norman Kaplan, DBRA/SBB; Dr. Richard Hudson, Dept. of Evolution and Ecology, University of California, Irvine, CA; Dr. Martin Kreitman, Department of Biology, Princeton University

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Eukaryotic Gene Structure and Function Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.00

## PROFESSIONAL:

1.0

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary focus of this project is the investigation of the relative roles of mutation, recombination, genetic drift and natural selection in shaping the levels of genetic variation observed at the DNA level. Several experiments address the fundamental question: what is the quantity and quality of molecular population genetic variation? To obtain a general answer many loci (white, yellow to achete, g-6-phd, forked, vermilion, suppressor of forked and zeste) in natural populations of *Drosophila* have been surveyed. A specific question in these and comparative studies with other species is the consequence of large differences in the amounts of crossing over per kilobase on the molecular genetic variation. The experimental results in conjunction with theoretical studies suggest that reduced levels of DNA sequence polymorphism in chromosome regions where crossing over is reduced are caused by the "hitch-hiking" effect of rare selectively favored and linked mutants.

A new aspect of this program that grew out of previous work examining transposons in *D. ananassae* is the cytological study of male meiosis. This species shows regular meiotic crossingover in males, which is atypical of other closely related *Drosophila* species.

This project except for the cytological analysis of *D. ananassae* male meiosis was terminated in January 1990. Major aspects of this research continue to be investigated by the PI and others of this group in their new positions. This project is continuing as a collaboration between Dr. Langley and the Judd group at NIEHS (see Z01 ES 65037-06 LMG). The light and electron microscopic examination of meiosis in *D. ananassae* males has produced some figures of cells in early prophase. Detailed examination of the electron microscope pictures should answer questions about crucial structures such as the synaptonemal complex.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 61042-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Expression During Drosophila Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Abbott	Staff Fellow	LMG, NIEHS
Others:	Willie Gibson	Research Chemist	LMG, NIEHS
	Krista Cartledge	Biological Aid (SIS)	LMG, NIEHS
	Sonja Ford	Summer IRTA	LMG, NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Eukaryotic Gene Structure and Function Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.83

## PROFESSIONAL:

1.0

## OTHER:

1.83

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The long-term goal of this project is to study the genetic control of morphogenesis. Our approach is to identify and characterize genes whose products have roles in morphogenetic processes occurring during the embryonic and post-embryonic development of Drosophila melanogaster. The specific processes under investigation are: (1) the transformation of the head of the embryo into the anterior end of the larva, (2) the rotation of the male genital disc during the pupal stage, and (3) the development of the sex-combs on the first pair of legs of the adult male fly.

One of the genes currently being investigated is head involution defective (hid). Genetic studies involving recessive mutations of hid have revealed that its expression is initially required sometime during the first half of embryogenesis for the proper development of the anterior end of the larva. Post-embryonic hid expression is required for the rotation of the male genital disc and wing morphogenesis. Further investigation into the role of this gene will involve the use of cloned hid DNA. We have cloned 70kb of DNA in the chromosomal region in which hid is located and are now searching within this DNA for the gene.

In addition to the aforementioned work, we have recently recovered 11 mutations in X-chromosome genes which affect either the rotation of the male genital disc or disrupt the formation of the male sex-combs. We are now characterizing these mutations genetically to determine how many different genes have been mutated and the precise location of each of these genes on the X-chromosome.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65034-06 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Specificity of Spontaneous and Induced Mutation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. M. Schaaper Visiting Scientist LMG NIEHS

Others: R. L. Dunn Biologist LMG NIEHS  
R. Cornacchio Stay-In-School Employee LMG NIEHS

COOPERATING UNITS (if any)

M. Radman, Laboratory of Molecular Genetics, NIEHS,  
R. P. Fuchs, Institut de Biologie Moleculaire et Cellulaire, Strasbourg, France,  
R. G. Fowler, San Jose State University, San Jose, California

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

1.25

PROFESSIONAL

0.5

OTHER

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project the mechanisms of mutagenesis are investigated through a detailed study of its specificity. DNA sequence information is gathered on all the classes of mutations that occur: base substitutions, frameshifts, deletions, duplications, insertion elements, complex rearrangements, etc. These classes have their own dependence on the local DNA sequence and generally result from different mutational pathways. The specificity of mutation thus provides a way to analyze and separate the various components of mutation. We use the lacI gene of the bacterium E. coli as a mutational target. The gene codes for the repressor of the lac operon and forward mutations to lacI<sup>-</sup> are scored based on their constitutive expression of the operon. The lacI<sup>-</sup> genes (typically several hundreds at a time) are transferred by in-vivo recombination to a single-stranded (recombinant) phage vector and sequenced, producing the mutational spectrum of interest. Comparing spectra in strains affected in various DNA repair/replication pathways is a next important step. In case of defined enzymatic pathways, the spectra provide a direct correlation between mutational classes and their responsible pathways. In case of unknown pathways, the mutational specificity may provide new insights into the affected pathway. So far, we have determined the specificity of mutation in mutH, mutL, mutS, mutI and mutD and wild-type strains of E. coli and have gained insights into the specific contributions of DNA damage, DNA mismatch repair and exonucleolytic proofreading to mutation. In case of induced mutagenesis, the specificity of mutation is a tool to identify both the nature of the premutagenic lesions and the mechanisms by which these lesions are converted into mutations. An example of this is the specificity of mutagenesis by the chemical carcinogen N-acetoxyacetylaminofluorene, which has enabled us to formulate a molecular model delineating how a single lesion may create different mutations depending on the local DNA sequence.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 65036-06 LMG</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Gene Organization and Regulation in <u>D. melanogaster</u></b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	<b>B. H. Judd</b>	Head, EGSFS  LMG, NIEHS
Others:	<b>Patricia S. Davis</b> <b>Shu-Mei Huang</b> <b>Katherine M. Peterson</b>	Chemist Geneticist Biologist  LMG, NIEHS LMG, NIEHS LMG, NIEHS
COOPERATING UNITS (if any)		
LAB/BRANCH <b>Laboratory of Molecular Genetics</b>		
SECTION <b>Eukaryotic Gene Structure and Function Section</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS:  <div style="text-align: center;"><b>2.00</b></div>	PROFESSIONAL:  <div style="text-align: center;"><b>0.25</b></div>	OTHER:  <div style="text-align: center;"><b>1.75</b></div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The project is focused on studies of gene expression during development in <u>Drosophila melanogaster</u>. Mutations of the <u>white</u> locus, which encodes a product that has strong sequence similarity with ATP-binding membrane transport proteins including the product of the cystic fibrosis gene of humans, show a wide range of changes in expression and regulation. We have concentrated on a group that originated by insertion of the transposon BEL into the 5' region of the gene. Derivatives of the original mutant strain show unusual regulatory changes in <u>white</u> expression. The mutant effects are cis-acting but similar to trans-acting effects between paired alleles in homologous chromosomes. We are attempting to understand the basis of the transvection phenomenon through the study of the cis-acting mutants. We are also studying the developmental regulation of <u>white</u> expression in various tissues and organs to determine its function.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65037-06 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transposon - mediated chromosome instabilities in Drosophila

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. H. Judd	Head, EGSFS	LMG, NIEHS
Others:	Shu-Mei Huang	Geneticist	LMG, NIEHS
	C. H. Langley	Research Geneticist	LMG, NIEHS
	E. A. Goode-Montgomery	Geneticist	LMG, NIEHS

## COOPERATING UNITS (if any)

Dr. Johng K. Lim, Distinguished Professor of Biology  
University of Wisconsin, Eau Claire

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Eukaryotic Gene Structure and Function Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.3

## PROFESSIONAL:

0.3

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transposons have been shown to play major roles in spontaneous mutation in *Drosophila* and other eukaryotes. In chromosomes, the insertion or excision of these mobile elements disrupts gene function at the site of insertion or creates deletions if excision is not precise. Additionally, recombination between two copies of a transposon situated at different sites in a chromosome or in homologs produce a variety of chromosomal rearrangements depending on the orientation of the paired mobile elements. We are studying rearrangements produced by these types of ectopic recombination to determine the molecular structures at their breakpoint junctions and to learn more about how transposons mediate these types of asymmetrical exchanges. We are also studying an example of high mobility by the retrotransposon *gypsy* to try to determine what conditions mediate such bursts of amplification and insertion into new chromosomal sites.







## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65038-05 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Mutagenesis by Animal Cell DNA Polymerases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others:	D. C. Thomas	Senior Staff Fellow	LMG NIEHS
	A. Sugino	Visiting Scientist	LMG NIEHS
	D. C. Nguyen	Biologist	LMG NIEHS
	K. Bebenek	Visiting Associate	LMG NIEHS

## COOPERATING UNITS (if any)

Kathleen Downey, Univ. of Miami, Miami, FL  
Robert A. Bambara, University of Rochester, Rochester, NY

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.7

## PROFESSIONAL:

0.6

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Maintenance of the stability of genetic information requires the accurate synthesis of DNA. In animal cells, DNA synthesis is performed by five distinct classes of DNA polymerase,  $\alpha$ ,  $\beta$ ,  $\delta$ ,  $\epsilon$  and  $\gamma$ . Our objective has been to characterize the accuracy of DNA synthesis by each of these enzymes and to analyze the errors committed by each in an attempt to understand how mutation rates are controlled. A major focus during the past year has been examination of the fidelity of the three putative replicative DNA polymerases,  $\alpha$ ,  $\delta$  and  $\epsilon$ . Accomplishments include the following. We have determined of the detailed error specificity of the four-subunit DNA polymerase  $\alpha$ -DNA primase complex purified from yeast, designated Pol I. The analysis forms the basis for future work on this replicative polymerase, which is already in progress with less- (as well as more-) complicated forms of the enzyme. This study has also led to testable models for production of large and complex deletion errors and for frameshift errors at non-reiterated nucleotide positions. We have characterized a mismatch-specific exonuclease associated with a second polymerase from yeast, Pol II (the putative analogue to mammalian pol  $\epsilon$ ). We have examined the error specificity of DNA polymerase  $\epsilon$ , which contains an associated proofreading exonuclease activity, to determine the contribution of base-selectivity and proofreading to fidelity. Parallel studies have begun with DNA polymerase  $\delta$ , which also contains an associated exonuclease that may serve a proofreading function. Each of these enzyme characterizations is central to our attempts to describe the molecular details for the accurate replication of human genetic information. In order to better understand the effects of known mutagens and carcinogens on the fidelity of DNA synthesis, we intend to extend these types of analyses to DNA substrates that contain defined lesions.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65041-04 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Repair in Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. M. Clark Senior Staff Fellow LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

1

PROFESSIONAL

1

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Errors made during DNA synthesis contribute significantly to the burden of spontaneous mutagenesis in most organisms. Cells have evolved complex mechanisms for correcting such errors, presumably to reduce the mutational load to tolerable levels. One such pathway, mismatch correction, operates specifically upon mispairs formed by misinsertion of an incorrect nucleotide during DNA synthesis. Generalized mismatch repair in *E. coli* is initiated by the product of the *mutS* gene, a protein that recognizes and binds to mispairs in DNA. Cells that carry mutations in the *mutS* gene show elevated rates of spontaneous mutagenesis.

Other laboratories have recently identified a possible mammalian analog of *mutS* on the basis of sequence similarity between the *mutS* gene and cDNAs derived from human and rodent cells. Mammalian cell lines in which the genomic DNA corresponding to the cDNA has been deleted might be expected to show an elevated rate of spontaneous mutagenesis. Spontaneous mutant frequencies were measured at the ouabain resistance locus in a chinese hamster cell line (DG22) carrying such a deletion and found to be indistinguishable ( $<1.7 \times 10^{-7}$ ) from wild type BH4 cells ( $<1.1 \times 10^{-7}$ ). The mutant frequency in BH4 cells was induced at least 100-fold to  $1.0 \times 10^{-5}$  when a transient nucleotide pool imbalance was created by treatment with 5 mM thymidine; surprisingly, the mutant frequency in DG22 cells was unchanged ( $<2.3 \times 10^{-7}$ ) by such treatment. Studies are currently in progress to determine the basis for this lack of mutagenic response to a presumed nucleotide pool imbalance.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65042-04 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Gene *uvrW* in Error-Prone Repair by Bacteriophage T4

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Head, Mutagenesis Section LMG NIEHS

Others: L. K. Derr Guest Worker LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

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INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

0.05

PROFESSIONAL

0.05

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. *uvrW* is a crucial but mysterious gene in the bacteriophage T4 EPR system. Mutations in *uvrW* depress recombination, increase killing and abolish mutagenesis by agents acting through EPR. Temperature-sensitive mutations of *uvrW* have been generated and characterized by mapping and complementation tests and their effects on survival, recombination and mutagenesis have been determined. A deletion mutation of *uvrW* has been engineered, providing a rigorously defined null allele. This work is now in press and the project is completed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65043-04 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Gene uvrX in Error-Prone Repair by Bacteriophage T4

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Head, Mutagenesis Section LMG NIEHS

Others: M. O. Rosario IRTA Fellow LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

0.30

PROFESSIONAL

0.30

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Most mutagens in most organisms act by triggering a process called error-prone repair (EPR). Such mutagens' primary action is to damage DNA in ways that block the progress of the DNA replication complex. EPR then facilitates damage bypass in a poorly templated (and therefore mutagenic) manner. The bacteriophage T4 uvrX gene plays a central role in EPR and also in recombination. Its product is a recombinase, a protein that catalyzes homologous strand exchange between DNA molecules. The specific role of this protein in EPR remains mysterious. Although several severe mutations of uvrX are only semilethal, there are hints that an even more drastic disruption of uvrX might be fully lethal. Therefore, mutations were introduced into early parts of the gene and the resulting mutants were examined for phenotype, including viability. These mutants were not substantially different from the canonical uvrX mutant. This work is now in press and the project is completed.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65045-04 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bacteriophage T4 rI Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Head, Mutagenesis Section LMG NIEHS

Others: D. C. Nguyen Chemist LMG NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.

0.25

PROFESSIONAL.

0.05

OTHER.

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mutagens contribute to the human burden of heritable birth defects and cancer and probably to heart disease as well. Bacteriophage T4 has been widely employed as a model system to analyze mechanisms of mutagenesis. One of the most common T4 mutation assays recognizes r (rapid lysis) mutants by their large, sharply edged plaques. Although the rII mutants are those most often subjected to further analysis, most mutagens produce more rI than rII mutants. Since little is known about the rI mutants, we have investigated their general properties. Mutations that produce the characteristic rI phenotype arise at two loci, one the classically described locus at about 60 kb on the standard map and another a locus at about 1600 kb. Point mutations at the 60-kb locus recombine inter se at low frequencies, suggesting a small gene; several are suppressed by unlinked but as yet unmapped suppressor mutations. The 1600-kb locus is being cloned and more closely mapped.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65046-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Accuracy of DNA Replication in vitro

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others: J. D. Roberts Senior Staff Fellow LMG NIEHS  
D. C. Thomas Senior Staff Fellow LMG NIEHS  
J. C. Boyer Staff Fellow LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

3.0

## PROFESSIONAL:

2.2

## OTHER:

0.8

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are interested in determining the mechanisms by which human cells control spontaneous and induced mutation rates. While DNA synthesis by purified DNA polymerases in vitro is not accurate enough to account for low spontaneous mutation rates in vivo, actual DNA replication involves the concerted action of a number of proteins. We have therefore been examining the fidelity of semiconservative bidirectional DNA replication by proteins present in extracts of human HeLa cells. The data obtained using mutagenesis vectors that monitor base substitution and frameshift fidelity indicate that replication is highly accurate. The major accomplishments for this year include completion of a detailed quantitative determination of replication error rates for a variety of errors. This analysis has shown that frameshift fidelity is dramatically greater than that of purified polymerases, providing the basis for complementation assays for identifying fidelity components. We have also used specialized vectors that place the replication origin on either side of the mutational target to determine the fidelity of leading versus lagging strand replication for minus-one base frameshifts and two transition mispairs. We have seen small differences (2- to 4-fold), but nothing dramatic yet. This observation is relevant the current model for a eukaryotic replication fork, which would predict that a difference should exist. Not unexpectedly, the current model is too simple. We have also demonstrated that efficient repair of mismatched base pairs occurs in the extract. Repair is strand-specific and directed by a nick. It requires the presence of the mismatch and is efficient for some mispairs and inefficient for others. ATP hydrolysis is required, repair can proceed in either direction, and the resynthesis tract size can be in excess of 1000 nucleotides. We intend to continue these studies, with emphasis on defining the details of mismatch repair, identifying fidelity factors, possibly including exonucelolytic proofreading activity, and examining molecular models for base-substitution and frameshift fidelity.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65047-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fidelity of Retroviral Reverse Transcriptases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others: J. D. Roberts Senior Staff Fellow LMG NIEHS  
K. Bebenek Visiting Fellow LMG NIEHS  
K. Eckert IRTA Fellow LMG NIEHS  
D. C. Nguyen Biologist LMG NIEHS

## COOPERATING UNITS (if any)

Samuel Wilson, Research Biochemist, LB, NCI  
Brendan Larder, Wellcome Research Laboratories, Kent, England

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL:

1.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A critical feature of the life cycle the human immunodeficiency virus (HIV-1) that causes Acquired Immunodeficiency Syndrome (AIDS) is its ability to generate diversity. HIV-1 has exceptionally high mutation rates within certain portions of its genome, permitting rapid evolution of new forms of the virus that are able to evade the host's immune response. In order to determine if errors committed by the viral reverse transcriptase could account for diversity in vivo, we had previously examined the accuracy of HIV-1 reverse transcriptase (RT) using in vitro fidelity assays and found this enzyme to be exceptionally error-prone. Sequence analysis of mutants resulting from in vitro synthesis demonstrates that the enzyme has unusual error specificity. Base substitution and one-base frameshift mutational hotspots are observed. The specificity and position of errors suggest that most mutational hot spots result from template-primer slippage. Using site-directed mutagenesis to alter the template DNA sequence for subsequent use in fidelity assays, we have obtained strong evidence that the frameshifts are indeed due to misalignment. Using a steady-state enzyme kinetic analysis of an exceptional base-substitution hot spot, we have also established equally strong support for a model for base substitutions generated by transient misalignment. Processivity analysis for the enzyme on the M13mp2 DNA template reveals strong termination at specific sites. Termination sites within homopolymer sequences correlate with frameshift mutational hot spots. Since these results suggest that the formation and/or utilization of misaligned template-primers is increased during the dissociation-reinitiation phase of the reaction, we are attempting to further examine this stage of the reaction. Our future work will continue to focus on elucidating the mechanisms responsible for the error-proness of HIV-1 RT. These studies may provide insights into the interaction of the enzyme's active site with its substrates and may be useful in designing RT-targeted drugs.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65048-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering DNA Polymerases to Probe Mutational Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. A. Kunkel Research Geneticist LMG NIEHS

Others: K. Bebenek Visiting Associate LMG NIEHS  
K. Eckert IRTA Fellow LMG NIEHS

## COOPERATING UNITS (if any)

Catherine M. Joyce, Yale University Medical School, New Haven, CT  
Samuel H. Wilson, LB, NCI, NIH, Bethesda, MD

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.1

## PROFESSIONAL:

1.1

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are using two DNA polymerases obtained by recombinant DNA technology, to examine the mechanisms and protein-DNA interactions that determine the fidelity of DNA synthesis. We have determined the fidelity of DNA synthesis catalyzed by the normal Klenow polymerase, by two mutant derivatives lacking proofreading exonuclease activity but having a normal protein structure, and by a protein that contains only the large polymerase domain. Measurements with the polymerases lacking an exonuclease show that the base-substitution fidelity of polymerization averages one error for each 10,000 to 40,000 bases polymerized, and can vary more than 30-fold depending on the mispair and its position. Steady-state kinetic measurements of selectivity at the insertion step by the exonuclease-deficient polymerase demonstrate differences in both the  $K_m$  and the  $V_{max}$  for incorrect versus correct nucleotides. Exonucleolytic proof-reading by the wild type enzyme improves the average base-substitution fidelity by 4- to 7-fold, reflecting efficient proofreading of some mispairs and less efficient proofreading of others. The wild-type polymerase is highly accurate for minus-one-base frameshift errors, with an error rate of  $\leq 10^{-4}$ . The exonuclease-deficient polymerase is less accurate, suggesting that proofreading also enhances frameshift fidelity. Even without a proofreading exonuclease, Klenow polymerase has high frameshift fidelity relative to several other DNA polymerases. Upon removal of the small domain, the large polymerase domain was found to have altered fidelity for several classes of mutations. The fidelity results have also permitted the examination of a model to explain the production of minus-one base frameshift errors at non-reiterated base sequences. We have also established the fidelity of the thermostable Taq polymerase used in polymerase chain reactions (PCR), using various reaction condition, including changes in temperature, pH, relative and absolute dNTP concentration and  $MgCl_2$  concentration. These studies define high fidelity conditions that should be useful for genetic applications of DNA amplified by PCR.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65050-04 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

## Molecular Analysis of Deletion Mutations in Chinese Hamster Ovary Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. R. Tindall Senior Staff Fellow LMG NIEHS

Others: R. A. Whitaker Biologist LMG NIEHS

COOPERATING UNITS (if any) Dr. Miroslav Radman, Inst. Jacques Monod, Paris-Cedex, France  
Dr. Donald Kufe, Harvard Medical School, Dana Farber Cancer Institute, Boston, MA  
Dr. William D. Caspary, Experimental Carcinogenesis and Mutagenesis Branch, DTRT, NIEHS, Research Triangle Park, NC

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL

0.4

## OTHER

0.2

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Chinese hamster ovary (CHO) cell line, AS52, carries a single functional copy of the bacterial *gpt* gene stably integrated into the CHO genome. Mutations at *gpt* are recovered as 6-thioguanine resistant colonies and may arise in these cells as a result of the loss of functional *gpt* sequences through i) intra-chromosomal deletion, ii) mitotic recombination or iii) gene conversion. The site of integration of the *gpt* locus appears to allow the recovery these complex mutations where such events will be conditionally lethal at the analogous hemizygous X-linked *hprt* locus. Thus, AS52 cells are sensitive to induced mutagenesis by a variety of clastogens which are often classified as nonmutagens in other assays. Two agents under study are 5-azacytidine (5AC) and 1-β-D-arabinofuranosylcytosine (ara-C). Neither 5AC nor ara-C are mutagenic at the *hprt* locus but both are potent mutagens at the *gpt* locus in AS52 cells. Ara-C has been demonstrated to generate deletions or recombination/gene conversion events and efforts are underway to define the spectrum of mutations induced by 5AC. We are continuing to refine the AS52 cell system to provide a more precise description of complex mutations. Efforts are underway to evaluate the requirement for perfect sequence homology and/or a role for mismatch repair in the regulation of mitotic recombination in mammalian cells. Similar studies in prokaryotes and lower eukaryotes have implicated mismatch repair in the regulation of recombination. These studies have direct bearing on the mechanisms by which mutations are induced by agents that saturate or inhibit mismatch repair. Such agents may be particularly recombinogenic and the AS52 cell line may be uniquely capable of detecting these events. Finally, we are continuing to use the *mxs*-system to isolate deletion endpoints for molecular characterization from lambda genomic libraries derived from selected AS52 mutants.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER <b>Z01 ES 65051-04 LMG</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Molecular Analysis of Point Mutations in Chinese Hamster Ovary Cells</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <b>PI: K. R. Tindall Senior Staff Fellow LMG NIEHS</b>		
<b>Others: R. A. Whitaker Biologist LMG NIEHS</b>		
COOPERATING UNITS (if any) <b>Dr. Robert W. Tuveson, Department of Microbiology, University of Illinois, Urbana, IL 61801</b>		
LAB/BRANCH <b>Laboratory of Molecular Genetics</b>		
SECTION <b>Mutagenesis Section</b>		
INSTITUTE AND LOCATION <b>NIEHS, NIH, Research Triangle Park, North Carolina 27709</b>		
TOTAL MAN-YEARS <b>1.3</b>	PROFESSIONAL <b>0.5</b>	OTHER <b>0.8</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             We use the Chinese hamster ovary (CHO) cell line (AS52) with a single copy of the bacterial <u>gpt</u> gene stably integrated into the genome to study point mutational changes in mammalian cells. Mutants arise as 6-thioguanine resistant (6TGR) colonies and mutant sequences are recovered using the polymerase chain reaction (PCR) followed by DNA sequence analysis to generate mutational spectra. We have identified a specific 3-base deletion represented in approximately 30% of the spontaneous AS52 mutants analyzed. However, in parallel studies at the <u>gpt</u> locus in <u>E. coli</u>, this 3-base deletion has not been observed. Differences between eukaryotic and prokaryotic DNA metabolism and/or chromosome structure may account for this striking difference in spectrum. Based on the specificity of this 3-base deletion in mammalian cells, the DNA sequences affected and the high frequency of this event, we suspect the involvement of eukaryotic DNA topoisomerase I. Experiments are underway using the topoisomerase I inhibitor, Camptothecin, as well as reversion analyses and targeted gene conversion in AS52 cells to evaluate the mechanistic basis of this deletion. In addition, we are generating comparative induced mutational spectra using the <u>E. coli</u> and AS52 cell systems. Evaluation of mutations induced by both UV-irradiation and H<sub>2</sub>O<sub>2</sub> are currently in progress. Finally, we are in the process of modifying the AS52 cell system to yield an accelerated phenotypic expression time and, therefore, more rapid generation of independent mutants for DNA sequence analysis.           </p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 65052-04 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Use of Retroviral Vectors in the Analysis of Mutations in Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: K. R. Tindall Senior Staff Fellow LMG NIEHS

Others:

COOPERATING UNITS (if any)

Dr. Larry Boone, Burroughs Wellcome Co., Research Triangle Park, NC  
Dr. Robert Langenbach, ECMB, DTRT, NIEHS, Research Triangle Park, NC

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Mutagenesis Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

0.1

PROFESSIONAL

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are interested in the effect of chromosomal position on the frequency and types of mutations observed using a target gene (gpt) integrated at different chromosomal sites in human cells. For these studies, we are using retroviral vectors that carry and express both the bacterial gpt and neo genes to construct human HT1080 cell lines with single copy integrations. A limitation to the general use of retroviral vectors in gene transfer studies is that some cells are resistant to retroviral infection. Therefore, to facilitate the application of retroviral vector technology to a variety of cell lines, we have devised a system that utilizes cationic liposomes (Lipofectin) for the delivery retroviral capsids. We have demonstrated that this approach allows one to bypass the normal receptor mediated route of infection allowing gene transfer to cell lines that either lack appropriate receptors or carry endogenous retroviruses that block the surface membrane receptors. For our mutagenesis studies, we have chosen a retroviral vector which allows the regulation of gpt gene expression using the human metallothionein (MTII) promoter. Evidence is accumulating to suggest that DNA repair may be more rapid in transcriptionally active regions of the genome and that the transcribed DNA strand may be repaired more rapidly than the nontranscribed strand. Thus, we hope to assess both the influence of DNA repair using a transcriptionally active or inactive target gene (gpt) as well as the effects of chromatin structure on mutagenesis. These studies are intended to provide a data base for using retroviral vectors to assess the role of human DNA repair pathways on mutational spectra generated in human repair deficient cell lines.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65053-03 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

DNA Sequence Characterization of Bacteriophage T4 rII Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Head, Mutagenesis Section LMG NIEHS

Others: M. C. Kricker Staff Fellow LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.20

## PROFESSIONAL:

0.20

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are using the bacteriophage T4 rII system as a model to explore mechanisms of DNA damage and mutagenesis. Because traditional DNA sequencing methods for analyzing the molecular nature of rII mutations are laborious and slow, we are developing methods based on genomic sequencing. With this method, important classes of mutations will be examined for their sequence changes. For instance, even mild heat damages DNA and could, if not repaired, produce on the order of 100 mutations per diploid human cell per day. Earlier studies showed that heat induces both transitions and transversions at G:C base pairs in phage T4. Genetic studies suggested that the main heat-induced transversion pathway is G:C to C:G but did not exclude G:C to T:A. We have shown that the actual mispairing of heat-damaged guanine (G\*) is G\*:A. This work has now been published and the project is completed.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65054-03 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Invariant Per-Genome Mutation Rates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake

Head, Mutagenesis Section

LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the late 1960s the then-available data from several laboratories suggested that microbes exhibited a constant forward mutation rate of about 0.003 per genome per replication; genome sizes varied by about 1000-fold, and so, inversely, did mutation rates per base pair per replication. This observation suggested that mutation rates had evolved to an optimum that was surprisingly constant among diverse organisms. Since then, the published data base has improved for many organisms and the invariance of per-genome mutation rates appears not only still to be the norm, but to extend all the way from a bacteriophage containing single-stranded DNA to microbial eukaryotes. However, RNA viruses and metazoans appear to possess mutation rates quite different from those of DNA-based microbes.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65055-02 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bacteriophage T4 Antimutator Mutations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. W. Drake Head, Mutagenesis Section LMG NIEHS

Others: D. C. Nguyen Chemist LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Antimutator mutations reduce spontaneous (and sometimes induced) mutation rates and are therefore of interest both for understanding mechanisms of spontaneous mutation and for finding ways to reduce mutation rates generally, and thus to reduce the incidence of diseases of mutational origin. We long ago discovered several antimutator alleles among temperature-sensitive mutations in the DNA polymerase gene of bacteriophage T4. These antimutators strongly reduced mutation rates along certain pathways, such as A:T to G:C, but were then found to have little effect on other pathways and even to act as mutator mutations on yet other pathways. We therefore initiated a search for generalized DNA polymerase antimutator mutations, defined as mutations that reduce mutation rates measured in a large target representative of the genome as a whole. None were found and this project is completed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65056-02 LMG

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evolution of the T-Even Bacteriophage tRNA Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: M. C. Kricker Staff Fellow LMG NIEHS

Others: J. W. Drake Head, Mutagenesis Section LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS.

0.50

## PROFESSIONAL.

0.50

## OTHER.

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transfer RNAs (tRNAs) are used as primers for reverse transcription during retroviral infection and may incorporate into the retroviral genome by illegitimate recombination. I am studying the tRNA gene cluster of the T-even bacteriophages as a model to investigate how they were incorporated into the viral genome. The tRNA genes vary widely among the T-even bacteriophages and have some features resembling reverse-transcribed mobile genetic elements. Additionally, each T-even phage expresses a unique set of tRNAs. The following questions will be explored. Are the tRNA genes mobile elements? Do they transpose via RNA intermediates? Is there sequence specificity at sites of loss or acquisition of tRNA genes? Is there illegitimate transfer of tRNA genes among the T-even bacteriophages or between them and other species? These questions will be approached by sequencing the tRNA gene clusters of phages T2, T4, and T6 to determine whether the tRNA genes are processed versions of tRNAs and to identify sites of loss or acquisition of tRNA genes. Genetic methods will be used to ask if these tRNA genes can transpose.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65057-02 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Mismatch Repair in Mutagenesis and Recombination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. Radman Visiting Scientist LMG NIEHS

Others: R. M. Schaaper Visiting Scientist LMG NIEHS  
K. R. Tindall Senior Staff Fellow LMG NIEHS  
M. A. Resnick Head, YG/MB Group CTGB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.5

## PROFESSIONAL

0.5

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Mismatch repair is a set of enzymological systems, encoded by numerous genes, that detect DNA base pair mismatches and convert them to standard base pairs. In one mode, mismatch repair examines freshly replicated DNA, detects mismatches of mutational origin, determines which is the wrong (progeny strand) base, and converts it to the correct base. In another guise, mismatch repair examines the hybrid regions of newly recombined DNA molecules, detects mismatches and acts to homogenize them in ways as yet poorly understood. Mismatch repair occurs in at least several bacteria, in yeast and in mammalian cells, and is probably ubiquitous. It constitutes a major barrier to spontaneous and induced mutation and to certain kinds of genetic recombination. A general theory has been developed to explain how mismatch repair may protect against illegitimate recombination in mitosis and meiosis. This work is being prepared for publication and the project is completed.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 65058-01 LMG

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of Stable Gene Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. C. Kricker Staff Fellow LMG NIEHS

Others: J. W. Drake Head, Mutagenesis Section LMG NIEHS  
M. Radman Visiting Scientist LMG NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular Genetics

## SECTION

Mutagenesis Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.95

## PROFESSIONAL

0.95

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The human genome harbors about one million interspersed repetitive sequences and many gene families, affording a myriad of opportunities for illegitimate recombination which can generating deletions, duplications, translocations and other genetic rearrangements, as well as reduction to deleterious homozygosity in mitosis. Yet mammalian genomes are remarkably stable. Are there specific molecular mechanisms in mammals and other higher eukaryotes that impede illegitimate recombination or mobile element transposition? Experiments with *E. coli* indicate that formation of heteroduplex recombination intermediates can be aborted due to the presence of only a few mismatches. Studies of *Neurospora* and *Ascomobolus* suggest that repeated sequences are preferentially methylated at cytosines, and 5-methylcytosines suffer deamination to thymidine, forming mutational hotspots. Methyl-directed mutation in these sequences has been designated as ripping (repeat-induced point mutations). Thus, divergence between repeated sequences could be generated via high-frequency methylation of cytosine, providing a mechanism to specifically inhibit recombination between multicopy sequences and to inactivate transposable elements by mutation. In higher eukaryotes methylation occurs most frequently at CpG dinucleotides. We are determining by computer analysis whether CpG dinucleotides are less frequent in gene families than in single-copy genes, and whether CpG dinucleotides undergo transition to TpG as would be expected if their cytosine residues were methylated and subsequently deaminated. Additional experiments will include sequencing of SV40 transgenes introduced into mice to determine if ripping occurs when multiple rather than single copies of genes have been introduced. These studies have significance for the expression of foreign genes in higher eukaryotes and for strategies of gene therapy in genetic disorders.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70090-07 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine and Neurochemical Regulation of Gonadal Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Negro-Vilar	Chief	LMIN	NIEHS
Others:	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	W. C. Wetsel	Senior Staff Fellow	LMIN	NIEHS
	F. Lopez	Visiting Scientist	LMIN	NIEHS
	M. Valenca	Guest Researcher	LMIN	NIEHS
	M. Wisniewski	Biologist	LMIN	NIEHS
	T. Flack	Chemist	LMIN	NIEHS
	T. Ionue	Visiting Fellow	LMIN	NIEHS

COOPERATING UNITS (if any)

University of North Carolina, Department of Anatomy, Chapel Hill, NC; University of Pécs, Department of Anatomy, Pécs, Hungary

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

1.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

The neuropeptide luteinizing hormone-releasing hormone (LHRH) is the prime regulator of gonadal function in vertebrates. Studies on the distribution of neurons containing pro-LHRH peptides have provided very useful information about the anatomical and functional arrangement of the LHRH network. Additional studies evaluating the expression, distribution and secretion of pro-LHRH peptides indicated that gonadal steroids can profoundly affect these parameters and thereby influence the overall activity of LHRH neurons. We also presented direct evidence that the LHRH neuronal system can "auto-regulate" its own activity, providing a functional correlate to the anatomical studies describing recurrent axon collaterals in LHRH neurons. This auto-regulatory mechanism may play a key role in determining a coordinated pulsatile or rhythmic LHRH neuronal activity. Using an *in vitro* system developed in our laboratory, we have performed an extensive characterization of the major neurotransmitters (norepinephrine, dopamine opioid peptides, GABA, etc.) regulating LHRH secretion, and of important internal (gonadal steroids and peptides, lactation, etc.) and environmental (stress, neurotoxins) factors affecting the interaction between neurotransmitters and the LHRH neurons. In many cases, these *in vitro* studies were conducted in parallel with *in vivo* paradigms, to obtain a direct estimation of changes in LHRH secretion and function *in vivo*. Steroids play a major role in maintaining the secretory capacity of the LHRH neuron, an effect which appears to be mediated by interneurons rather than by direct actions at the LHRH neuron. The *in vitro* model allowed us to characterize the role of  $Ca^{2+}$ , arachidonate metabolites ( $PGE_2$  and different lipoxigenase metabolites) and protein kinase C activation on the regulation of pro-LHRH peptide(s) secretion from nerve terminals. These studies should advance our understanding of the complex interactions between central neurotransmitter systems and internal or external environmental factors influencing reproductive functions.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 70092-07 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Mechanisms Mediating Peptide Hormone Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Negro-Vilar	Chief	LMIN	NIEHS
Others:	F. Lopez	Visiting Scientist	LMIN	NIEHS
	M. D. Culler	Senior Staff Fellow	LMIN	NIEHS
	W. Wetzel	Senior Staff Fellow	LMIN	NIEHS
	T. Inoue	Visiting Fellow	LMIN	NIEHS
	I. Wanderley	Guest Researcher	LMIN	NIEHS

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Endocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.8

PROFESSIONAL:

2.2

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

It is now well recognized that hypothalamic and pituitary hormones are secreted in a pulsatile pattern which is unique for each hormone and which may vary according to the physiological status of the subject. The evidence we have obtained supports the concept that the pulsatile secretory pattern contains encoded messages that convey the required inputs to elicit secretory responses and other important biological events, such as cell differentiation and even enhanced gene expression. It seems evident, therefore, that pulsatile hormone secretion represents a sophisticated, carefully regulated means of intracellular communication. We have evaluated the characteristics of the pulsatile pattern of secretion of most pituitary hormones and of some hypothalamic peptides as well. These studies indicate that several parameters of the pulsatile pattern can change during different physiological situations or after specific pharmacological interventions. Secretion of the neuropeptide LHRH into the hypophysial portal blood in intact animals occurs in a pulsatile fashion. Evaluation of the total amount (mass) of hormone secreted in each pulse (measuring area under the pulse) reveals that at least two distinct populations of pulses can be separated, i.e., "small" and "big" mass pulses. Orchidectomy results in an almost complete disappearance of "big mass" pulses. Testosterone replacement reestablishes the presence of large mass pulses. These observations are helping to re-define the established dogma of negative steroid feedback, into a new concept in which the steroids interact with neural structures to modify the pulse pattern of peptide release. This may be accomplished by establishing a functional neuronal network capable of generating a pulsatile pattern of LHRH secretion which can appropriately maintain pituitary-gonadal function. Additional studies on the pulsatile pattern of hormones under dual (stimulatory/inhibitory) control (such as prolactin) or under multifactorial neural regulation (ACTH) also provided very useful information about the encoding of signals on the pulsatile pattern which may contribute to the pleiotropic actions of these hormones.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 70096-06 LMIN
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Regulation of Pulsatile Gonadotropin Secretion		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	Michael D. Culler	Senior Staff Fellow LMIN NIEHS
Others:	Andres Negro-Vilar Carl Paschall	Chief Biologist LMIN NIEHS
<b>COOPERATING UNITS</b> (if any) Department of Anatomy, University of North Carolina, Chapel Hill, NC Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA		
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience		
<b>SECTION</b> Reproductive Endocrinology Section		
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unreduced type. Do not exceed the space provided.) <p>During the past year, we have examined the interaction of the gonadal peptide, inhibin, with the gonadal steroids as they modulate the basic brain-pituitary regulation of gonadotropin (LH and FSH) secretion. We found that inhibin can be demonstrated to suppress basal FSH secretion and all parameters of LH secretion in the intact, adult female rat but cannot be demonstrated to affect gonadotropin secretion in the intact, adult male rat. Destruction of testis Leydig cells with the selective toxicant, ethane dimethane sulfonate (EDS), revealed that Leydig cell influences, which include testosterone, can account for the total suppressive influence of the testes on all parameters of LH secretion. The Leydig cell influences suppress only basal FSH secretion, however, and only partially account for the total FSH-suppressing influence of the testes. In contrast to the intact rat, inhibin was demonstrated to account for the remaining suppressive influence of the EDS-treated testis on basal FSH secretion suggesting that the contribution of inhibin in the adult male rat is either masked by the larger Leydig cell influence or absent until activated by Leydig cell impairment. In concomitant studies, an effect of endogenous inhibin was also demonstrated in LHRH driven, juvenile male rhesus monkeys. Studies in the female rat have confirmed our previous findings that inhibin selectively suppresses only basal FSH secretion and demonstrated that pulsatile FSH secretion may be suppressed by estradiol. In addition, we have initiated <u>in vitro</u> studies examining pituitaries from rats in which the gonadal factors have been selectively neutralized in order to determine the affected gonadotropin secretion phase and intracellular messenger systems. Finally, we have initiated studies to characterize the different molecular weight species of inhibin and to relate the changing ratios of these forms with alterations in the reproductive state. The results from these and ongoing studies are dissecting the mechanisms by which the brain and gonads interact to control gonadotropin secretion and reproductive function.</p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 ES 90033-08 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Milk Bradykinin and Kininogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: William E. Wilson Research Chemist LMIN NIEHS

Others: L. H. Lazarus Research Chemist LMIN NIEHS  
M. Ott Biologist LMIN NIEHS  
A. Haugen Biologist LMIN NIEHS

COOPERATING UNITS (if any)

University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Peptide Neurochemistry Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Bradykinin's involvements in physiological phenomena such as blood clotting, vascular smooth muscle contractility, peripheral nociception, etc. have been extensively studied, however, involvement of this or structurally related kinins in other physiological functions has received little attention. Our recent finding that several kinins, including bradykinin, occur in bovine milk provided chemical evidence to rationalize earlier observations regarding the likely involvement of kininogens and/or kallikreins in postnatal development. Recent efforts to recover milk kininogens have permitted resolution of two soluble forms by isoelectric focusing, the low-pI and high-pI milk kininogens; a third form has also been recognized. The MW of the milk HMW kininogen appears to be around 68,000 and the pI is  $4.15 \pm 0.5$ ; by contrast, the MW of bovine plasma HMW kininogen is 76,000 and its pI is 4.6. Methods are being developed to facilitate recovery of pure bovine milk kininogens in order to permit (a) determination of structural difference(s) between milk and plasma kininogens and (b) to ascertain the chemical nature of kinins, other than bradykinin, which may occur in milk. The long-term goals are to develop methods which may be generally applied to isolation of tissue kininogens and to ascertain the potential physiological significance(s) of such molecules.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90034-07 LMIN
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990		
<b>TITLE OF PROJECT</b> (80 characters or less. Title must fit on one line between the borders.) Antigenicity of PHLIP-8 and Other Physiological Peptides		
<b>PRINCIPAL INVESTIGATOR</b> (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: William E. Wilson      Research Chemist      LMIN    NIEHS  Others: L. H. Lazarus      Research Chemist      LMIN    NIEHS		
<b>COOPERATING UNITS</b> (if any) University of North Carolina, Chapel Hill, NC		
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience		
<b>SECTION</b> Peptide Neurochemistry Group		
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709		
<b>TOTAL MAN-YEARS:</b> 0.1	<b>PROFESSIONAL:</b> 0.1	<b>OTHER:</b> 0.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK</b> (Use standard unexpanded type. Do not exceed the space provided.)  Efforts to elicit polyclonal antibodies to PHLIP-8 (the N-terminal octapeptide of rabbit stomach mucin is <Glu-Val-Asp-Pro-Asn-Ile-Gln-Ala-OH) have been unsuccessful; also, recent efforts to raise polyclonal antisera to bradykinin (Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg) have met with very limited success. Our experiences, as well as those of others with TRH (<Glu-His-Pro) and tuftsin (Thr-Lys-Pro-Arg), indicate that many small, physiologically important peptides are poor antigens when coupled to protein carriers using conventional techniques. As antisera to several of these peptides are required in other ongoing projects, efforts will continue to devise strategies to raise polyclonal and/or monoclonal antisera using a variety of peptide structural analogues as antigens in addition to a variety of proteins known to elicit immune system responsiveness.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90042-04 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990 TERMINATED May 15, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models of Neurodegenerative Processes Involving Cognitive and Motor Dysfunction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Hugh A. Tilson	Pharmacologist	LMIN	NIEHS
Others:	B. Rogers	Biologist	LMIN	NIEHS
	W. Zhang	Visiting Fellow	LMIN	NIEHS
	P. Tandon	Visiting Fellow	LMIN	NIEHS
	K. Nanry	Psychologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS
	L. Williams	Stay-in-Schooler	LMIN	NIEHS

COOPERATING UNITS (if any)

Duke University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurobehavioral Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

5.55

PROFESSIONAL:

2.60

OTHER:

2.95

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90043-05 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Zinc in Synaptic Transmission in the Hippocampal Formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Clifford L. Mitchell Pharmacologist LMIN NIEHS

Others: J. S. Hong Pharmacologist LMIN NIEHS  
J. McGinty Assoc. Professor East Carolina University

COOPERATING UNITS (if any)

Department of Anatomy, East Carolina University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurophysiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Several pieces of evidence suggest that endogenous opioids and zinc may interact to regulate neuronal excitability within the hippocampal formation. The purpose of this project is to conduct a systematic investigation into the effects of zinc on hippocampal neuronal excitability. The goal is to explain the nature of the effects of zinc and the mechanism(s) for its modulation of hippocampal excitability. First it was necessary to determine the manner in which zinc levels were to be altered. As an initial approach we chose to attempt to alter zinc levels by systemic administration of the intraviral zinc chelators, dithizone and diethyldithiocarbamate (DEDTC). The biological assay used was occurrence of wet dog shakes and seizures following subcutaneous administration of kainic acid (KA). Intraperitoneal injection of dithizone (12.5-100 mg/kg) or DEDTC (100-400 mg/kg) has a profound and dose related effect on the effects of KA. When given 15 minutes after the subcutaneous injection of KA, they markedly potentiate KA activity. They also produce a transient decrease in hippocampal levels of enkephalin and dynorphin. They also produce transient increases in the hippocampal levels of a number of amino acids (viz., taurine, glutamate, glutamine, and GABA). These effects are associated with reduced levels of hippocampal zinc (as measured by Timm staining of the hippocampus). Work in progress is investigating the effect of DEDTC on seizure activity elicited by electrical stimulation of the perforant path (the major input to the hippocampal formation). Preliminary evidence suggests that, like for KA, DEDTC enhances seizure activity induced by stimulation of the perforant path. It appears, then, that dithizone and diethyldithiocarbamate may prove to be useful tools for exploring the actions of zinc on the hippocampus. Other work in progress involves: (1) effects of injection of these agents locally in the hippocampus, and (2) examination of their electrophysiological effects on the hippocampus.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90044-05 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of Neuronal Function by Neuropeptides and Steroid Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Clifford L. Mitchell	Pharmacologist	LMIN	NIEHS
Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	C. W. Xie	Visiting Fellow	LMIN	NIEHS
	P. Lee	Visiting Associate	LMIN	NIEHS
	J. McGinty	Assoc. Professor	East Carolina University	

COOPERATING UNITS (if any)

Department of Anatomy, East Carolina University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurophysiology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Work in this laboratory has focused on the role of enkephalin and dynorphin in seizure activity and related sequelae. This work has implicated enkephalin as playing a major role in the elucidation of a phenomenon in rats known as "wet dog shakes" (WDS). This work has also implicated the dentate granule cells (DGCs) as being necessary for the elicitation of WDS at least with respect to induction by kainic acid or by stimulation of the perforant path (PP). We have demonstrated that stimulation of PP under conditions which elicit WDS produces a significant decrease in hippocampal levels of enkephalin and dynorphin. Moreover, intraventricular injection of either an opioid mu receptor (8-FNA) or delta receptor (ICI174864) antagonist reduced the number of WDS elicited by PP stimulation. These data provide the first evidence that endogenous opioids are released by PP stimulation and lend further support to the notion that they play a role in regulation of hippocampal excitability. We have also demonstrated that the opioid receptor antagonist, naltrexone, when injected directly into the ventral hippocampus, produces an elevation in the threshold for eliciting wet dog shakes. We have also demonstrated that destruction of dentate granule cells in the ventral, but not dorsal, hippocampal formation abolishes wet dog shaking induced by perforant path or intrahippocampal stimulation or by systemic administration of kainic acid. It has also been found that slices obtained from the ventral portion of the hippocampus have a lower threshold for epileptiform bursting induced by an opioid mu receptor than slices from the dorsal end. Thus, these studies clearly demonstrate differences between the ventral and dorsal portions of the hippocampus. This is of importance since most previous studies have viewed the hippocampus as being functionally homogeneous. We have also found that destruction of DGCs (which deplete the hippocampus of dynorphin) results in a marked lowering of the threshold for induction of seizure activity elicited by PP stimulation.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90045-05 LMIN	
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990			
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Studies on the Relationship between Opioid Peptides and Seizures			
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b> PI: Jau-Shyong Hong                      Pharmacologist                      LMIN                      NIEHS			
Others: P. Lee                      Visiting Associate                      LMIN                      NIEHS T. Xie                      Visiting Fellow                      LMIN                      NIEHS C.L. Mitchell                      Pharmacologist                      LMIN                      NIEHS			
<b>COOPERATING UNITS (If any)</b>			
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience			
<b>SECTION</b> Neuropharmacology Section			
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709			
<b>TOTAL MAN-YEARS:</b> 1.3		<b>PROFESSIONAL:</b> 1.3	
		<b>OTHER:</b> 0.0	
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
<b>SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)</b> <p>           The purposes of this project were: 1) to determine alterations in the metabolism of enkephalins and dynorphins in the limbic-basal ganglia regions after chemical- or electrically-induced seizures; 2) to study the possible roles of brain opioid peptides in seizure-induced changes in hippocampal excitability. Previous studies showed that stimulation of perforant path, which projects from the entorhinal cortex to the dentate gyrus of the hippocampus, elicits a behavioral response, wet dog shakes, and decreases the level of dynorphin in the hippocampus. This study examined the molecular mechanisms underlying the perforant path stimulation-induced reduction in dynorphin. It is well-documented that glutamate is a neurotransmitter released from the perforant path. We have reported that stimulation of perforant path increased the extracellular concentration of glutamate in the hippocampus measured by microdialysis technique in free moving rats. Thus, it is likely that glutamate may regulate the metabolism of dynorphin in the dentate granule cells. To test this possibility, we employed a glutamate antagonist, gamma-D-glutamylglycine (γ-DDG), which binds non-selectively to different glutamate receptor subtypes. Daily DGG pretreatment almost abolished WDS at control threshold intensities, and significantly inhibited stimulation-induced decrease of DYN-IR in both dorsal and ventral hippocampus. In situ hybridization using a <sup>35</sup>S-labeled oligodeoxyribonucleotide probe demonstrated a clear depletion of DYN mRNA signal in dentate granule cell layer of ACSF-treated animals. This depletion was completely prevented in DGG-treated rats. These data strongly suggest that glutamate as the endogenous transmitter at perforant synapses mediates stimulation-induced synaptic excitation and regulates the release and biosynthesis of dynorphin peptides in dentate granule cells. This project will be terminated September 30, 1990.         </p>			



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90049-04 LMIN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Gene Expression in Adrenomedullary Chromaffin Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. S. Hong Pharmacologist LMIN NIEHS

Others: M. K. Stachowiak	Senior Staff Fellow	LMIN	NIEHS
P. Hudson	Biologist	LMIN	NIEHS
R. Tuominen	Visiting Associate	LMIN	NIEHS
H. Ye	Guest Worker	LMIN	NIEHS
M. McMillian	Senior Staff Fellow	LMIN	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Neuropharmacology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.5

## PROFESSIONAL:

3.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goals of this project were: 1) to examine the nature of extra-cellular and intracellular signals controlling expression of tyrosine hydroxylase (TH), phenylethanolamine-N-methyl-transferase (PNMT), and proenkephalin (pEK) genes; 2) to determine whether these genes are differentially regulated; 3) to determine roles of transcription and post-transcriptional mechanisms in such regulations; and 4) to examine the possible role of nuclear oncogenes in coordinating the regulation of TH, PNMT, and pEK genes. Previous studies from our laboratory suggest that protein kinase C (PKC) is involved in the angiotensin II (AII) induced increase in the expression of genes encoding proenkephalin and catecholamine biosynthesizing enzymes in primary cultured bovine adrenal medullary (BAM) cells. The purpose of this study was to examine the effects of [Sar<sup>1</sup>]-AII (S<sup>1</sup>-AII), an AII agonist, on PKC activity in BAM cells. Short-term incubation with S<sup>1</sup>-AII produced a dose-dependent activation of PKC. The particulate PKC activity was significantly increased by 2 nM S<sup>1</sup>-AII after both short- and long-term incubation. A high concentration of S<sup>1</sup>-AII (200 nM) caused a translocation of PKC activity from cytosolic to particulate fractions after 10 min incubation with the translocation still observed after 18 hrs of continuous incubation. Sar<sup>1</sup>-Thr<sup>8</sup>-angiotensin II (S<sup>1</sup>-T<sup>8</sup>-AII), an AII-antagonist, inhibited the effect of S<sup>1</sup>-AII (20 nM) on PKC activity, suggesting a specific AII receptor-mediated effect. An increase in BAM cell particulate PKC immunoreactivity after 18 hr S<sup>1</sup>-AII treatment was observed in western-blot analysis of PKC immunoreactive protein (82 kD). The persistent activation of PKC seen in this study is consistent with our hypothesis that PKC may mediate the S<sup>1</sup>-AII induced increase in the expression of genes encoding proenkephalin and catecholamine synthesizing enzymes in BAM cells.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90050-04 LMN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Roles of Opioid Peptides in the Regulation of Hippocampal Excitability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Lee Visiting Associate LMN NIEHS

Others: Jau-Shyong Hong Pharmacologist LMN NIEHS  
P.M. Hudson Biologist LMN NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neuropharmacology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Roles of opioid peptides in the regulation of hippocampal excitability are under intensive study after the discovery of endogenous opiates in the brain. Intraventricular administration of opioid peptides elicited epileptiform discharges and wet dog shakes (WDS) in rats, however, no behavioral convulsion was observed. We have shown that a single unilateral injection of specific mu opioid receptor agonists into the ventral hippocampus, but not into the dorsal hippocampus or other brain regions, resulted in a dose-dependent increase in the frequency of convulsions and wet dog shakes. We also demonstrated that these opioid-induced behavioral changes were mediated exclusively by mu but not delta or kappa opioid receptors in the ventral hippocampus. The disparity between the ventral and dorsal hippocampus in seizure sensitivity to mu opioid receptor agonists could be due to differences either extrinsic or intrinsic to the hippocampus. The latter possibility was tested in this study with an in vitro method using dorsal and ventral hippocampal slices from the same rat. Paired dorsal and ventral hippocampal slices were perfused with [NMe-Phe<sup>3</sup>-D-Pro<sup>4</sup>]morphiceptin (PL017), a specific mu opioid receptor agonist. Application of 0.05  $\mu$ M PL017 produced triggered and spontaneous bursting in 20% of ventral hippocampal slices, but no such effect was observed in dorsal hippocampal slices. At 0.5  $\mu$ M PL017, 80% of ventral slices developed spontaneous bursting, whereas only 10% of dorsal slices had spontaneous bursting. The addition of 0.1  $\mu$ M naloxone prior to or after PL017 inhibited the triggered response and reduced the frequency of the spontaneous bursting. These results suggest that the ventral hippocampus has a higher susceptibility to PL017-induced epileptiform bursting, and this effect is mediated, at least in part, through mu opioid receptors. Further studies are planned, by using hippocampal primary cell culture as a tool, to determine molecular mechanisms of opiate-induced excitability in the hippocampus. This project will be terminated September 30, 1990.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90051-03 LMIN
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990 <b>TERMINATED MAY 15, 1990</b>		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Brainstem and Spinal Cord Modulation of Neurological Motor Dysfunction		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</b>		
PI:	Hugh A. Tilson	Pharmacologist      LMIN      NIEHS
Others:	K. Nanry	Psychologist      LMIN      NIEHS
	C. Hamm	Electronics Engineer      LMIN      NIEHS
<b>COOPERATING UNITS (if any)</b>		
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience		
<b>SECTION</b> Neurobehavioral Section		
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.60	0.25	0.35
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)</b> <div style="height: 300px; border: 1px solid black;"></div>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90052-02 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990 TERMINATED MAY 15, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Compensation and Recovery of Function in the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	William R. Mundy	Staff Fellow	LMIN	NIEHS
Others:	H. Tilson	Pharmacologist	LMIN	NIEHS
	C. Watters	Stay-in-Schooler	LMIN	NIEHS
	K. McDaniel	Biologist	LMIN	NIEHS
	R. McLamb	Biologist	LMIN	NIEHS
	C. Hamm	Electronics Engineer	LMIN	NIEHS
	S. Barone	Guest Worker	LMIN	NIEHS
	M. Bonner	Guest Worker	LMIN	NIEHS

COOPERATING UNITS (if any)

East Carolina University

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Neurobehavioral Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

5.60

PROFESSIONAL:

2.15

OTHER:

3.45

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90053-03 LMIN
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Neuropeptides: Molecular Mechanism of Action		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</b> PI:        Lawrence H. Lazarus                      Research Chemist                      LMIN                      NIEHS  Others:   William E. Wilson                      Research Chemist                      LMIN                      NIEHS		
<b>COOPERATING UNITS (if any)</b> S. Salvadori, University of Ferrara, Italy; R. de Castiglione, Farmitalia Carlo Erba, Milan, Italy		
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience		
<b>SECTION</b> Peptide Neurochemistry Group		
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709		
<b>TOTAL MAN-YEARS:</b> 1.0	<b>PROFESSIONAL:</b> 1.0	<b>OTHER:</b> 0.0
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)</b> The initial phases of these studies involved the in vivo central administration of neuropeptides on gastric acid secretion. The high affinity $\mu$ selective opioid heptapeptide dermorphin produced marked suppression of the secretion of gastric acid in conscious rats. This effect was blocked by the prior administration of indomethacin, which suggests that the synthesis of prostaglandins is required for the inhibitory action of neuropeptides on gastric secretion. These data are in accord with clinical studies which showed that individuals being treated with morphinomimetic analgesic drugs exhibit a lower incidence of ulcers in the mucosal lining of the stomach. These results led to the second phase: receptor analyses of the interaction of dermorphin with brain membrane binding sites. The binding data provided information on the formulation of a new model for the receptor interaction with $\mu$ selective opioid peptides: (1) deletion of Tyr <sup>8</sup> or replacement by a hydrophilic residue, disrupted recognition at both $\mu$ and $\delta$ receptor sites; (2) blockage of the functional groups on Tyr <sup>1</sup> similarly reduced binding; (3) addition of hydrophobic protective groups on Ser <sup>7</sup> increased the affinity to $\mu$ and $\delta$ sites, but decreased $\mu$ selectivity; and (4) the requirement for a D configuration about the $\alpha$ carbon of residue 2 and amidation of the C-terminal residue is essential. This model proposes that the $\mu$ receptor accommodates two binding pockets: a Ty site for the stacked Tyr <sup>1</sup> /Tyr <sup>5</sup> residues and a Py site for the Phe <sup>3</sup> residue, which extends outward from the peptide backbone. Reversal of the Phe <sup>3</sup> -Gly <sup>4</sup> dipeptide region in dermorphin to the Gly <sup>3</sup> -Phe <sup>4</sup> sequence in enkephalin drastically diminished $\mu$ selectivity. Thus, the N-terminal sequence, H-Tyr-D-Ala-Phe-Gly, particulates in a $\beta$ -turn through internal hydrogen bonds and constitutes the message domain of the peptide.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90054-03 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (50 characters or less. Title must fit on one line between the borders.)

Regulation of Biosynthesis, Processing and Secretion of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: William C. Wetzel Senior Staff Fellow LMIN NIEHS

Others: Andres Negro-Vilar Chief LMIN NIEHS  
Hector Rivera Biological Lab Technician LMIN NIEHS  
Gail Wisniewski Biologist LMIN NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.1

PROFESSIONAL:

1.0

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

Peptides represent a major mode of chemical transmission in the nervous and endocrine systems. Our studies have focused upon the LHRH prohormone as a model system to study the regulation of biosynthesis, processing, and secretion of neuropeptides. Previously, we have described the metabolic pathway for the pro-LHRH peptide in rat brain and have shown that gonadal steroids influence the biosynthesis and secretion of the major pro-LHRH products: LHRH and GAP. More specifically, gonadal steroids selectively affect both protein kinase C (PKC) activity and PKC-coupled secretion of LHRH and GAP. Recently, we have obtained a neuronal cell line, developed at the Salk Institute, which secretes LHRH-like peptides. When materials in cell extracts or media are separated by size-exclusion chromatography and assayed by radioimmunoassay, these cells are found to biosynthesize the pro-LHRH peptide, to process this precursor to LHRH and GAP, and to secrete these peptides into the media in response to PKC activation and  $[K^+]$ -depolarization. When these cells are examined by EM, both LHRH and GAP immunoreactivity are co-localized in the same secretory vesicle. These cells form synapses and tight junctions with each other. These structural features provide a functional basis for the rhythmic and pulsatile secretion of LHRH that we have observed. These findings demonstrate that the LHRH neuron contains a pulse generator which may entrain the gonadotrophs of the pituitary to secrete LH in a pulsatile manner. Future studies will examine in detail the molecular mechanisms which regulate biosynthesis of the pro-LHRH peptide, the steps involved in its processing, and the various pathways of its secretion.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90055-02 LMIN																								
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990																										
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Hypothalamic Control of the Anterior Pituitary, Morphological Aspects																										
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</b> <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Istvan Merchenthaler</td> <td style="width: 40%;">Visiting Scientist</td> <td style="width: 20%;">LMIN</td> <td style="width: 20%;">NIEHS</td> </tr> <tr> <td colspan="4" style="padding-top: 10px;">Others: A. Negro-Vilar</td> </tr> <tr> <td></td> <td>Chief</td> <td>LMIN</td> <td>NIEHS</td> </tr> <tr> <td>Zsolt Liposits</td> <td>Visiting Scientist</td> <td>LMIN</td> <td>NIEHS</td> </tr> <tr> <td>David Lennard</td> <td>Biologist</td> <td>LMIN</td> <td>NIEHS</td> </tr> <tr> <td>JoAnne Reid</td> <td>Biologist</td> <td>LMIN</td> <td>NIEHS</td> </tr> </table>			PI: Istvan Merchenthaler	Visiting Scientist	LMIN	NIEHS	Others: A. Negro-Vilar					Chief	LMIN	NIEHS	Zsolt Liposits	Visiting Scientist	LMIN	NIEHS	David Lennard	Biologist	LMIN	NIEHS	JoAnne Reid	Biologist	LMIN	NIEHS
PI: Istvan Merchenthaler	Visiting Scientist	LMIN	NIEHS																							
Others: A. Negro-Vilar																										
	Chief	LMIN	NIEHS																							
Zsolt Liposits	Visiting Scientist	LMIN	NIEHS																							
David Lennard	Biologist	LMIN	NIEHS																							
JoAnne Reid	Biologist	LMIN	NIEHS																							
<b>COOPERATING UNITS (if any)</b> University of North Carolina, Department of Cell Biology and Anatomy, Chapel Hill, NC; University of Pecs, Department of Anatomy, Pecs, Hungary																										
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience																										
<b>SECTION</b> Functional Morphology Section																										
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709																										
<b>TOTAL MAN-YEARS:</b> 3.3	<b>PROFESSIONAL:</b> 1.3	<b>OTHER:</b> 2.0																								
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																										
<b>SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)</b>  <p>During the past year, we concentrated our work on studying (1) the co-localization of LHRH and galanin in a subset of hypothalamic neurons, (2) the identification of hypophysiotropic neurons, and (3) the immunocytochemical characterization of LHRH cell lines derived from transgenic mice. (1) We have demonstrated for the first time that LHRH and galanin are co-localized and, moreover, that the degree of co-localization is estrogen-dependent. While in the male approximately 20% of the LHRH cells contain galanin, in the female rat, sacrificed on proestrous, the degree of co-localization exceeds 60%. Galanin is not only co-localized with LHRH but seems to innervate LHRH perikarya. These anatomical and functional connections support our observations that galanin is a potent LHRH releasing factor. (2) With a combination of retrograde tracing and immunocytochemistry, a technique suitable for the chemical and anatomical identification of hypophysiotropic neurons was developed. Fluoro-Gold, injected peripherally, has been used as retrograde tracer, the neuropeptide content of the retrogradely labeled perikarya can be determined by immunocytochemistry. Using this approach we have shown that the major sources of the hypophysiotropic galanin neurons are the arcuate and paraventricular nuclei. TRH and CRF neurons are derived from the paraventricular nucleus, while neurotensin neurons originate exclusively from the arcuate nucleus. (3) We have shown by light and electron microscopic immunochemistry that the LHRH cell lines derived from transgenic mice produce both LHRH and GAP. Both peptides are present in the same secretory granule. The cells form extensive synaptic connections with each other, thereby providing the morphological basis for a synchronized secretory activity. The results from these and ongoing studies provide the morphological support for understanding the mechanisms by which the hypothalamus controls the function of the anterior pituitary.</p>																										



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90056-02 LMIN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Hippocampal Opioid Peptides by Excitatory Amino Acids and Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Jau-Shyong Hong	Pharmacologist	LMIN	NIEHS
Others:	P. Lee	Visiting Associate	LMIN	NIEHS
	C.L. Mitchell	Pharmacologist	LMIN	NIEHS
	L. Thai	Lab. Technician	LMIN	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Neuropharmacology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several lines of evidence suggest that endogenous opioid peptides, enkephalin and dynorphin play important roles in modulating the excitability of the hippocampus. However, the information regarding the regulation of these two opioid peptides has been lacking. The purpose of this project was to examine the molecular mechanisms of the expression of enkephalin and dynorphin in the hippocampus by excitatory amino acids or hormones, such as glucocorticoids. Previous results from our laboratory indicate that stimulation of perforant pathway, which innervate dentate granule cells, elicits differential effects on the hippocampal levels of opioid peptides: increase in enkephalin, but decrease in dynorphin. To study the neurotransmitter(s) which regulate the metabolism of dynorphin in the dentate granule cells, two sets of experiments were performed. First, to examine the possibility that glutamate, which releases from the perforant path following stimulation, may be responsible for the down regulation of dynorphin, a glutamate receptor blocker, gamma-D-glutamylglycine (γ-DGG) was employed. Daily DGG pretreatment significantly inhibited stimulation-induced decrease in dynorphin content in both dorsal and ventral hippocampus. This result was further supported by *in situ* hybridization which measures the abundance of pro-dynorphin mRNA levels. These data strongly suggest that glutamate as the endogenous neurotransmitter at perforant synapses mediates stimulation-induced synaptic excitation and regulates the release and biosynthesis of dynorphin in dentate granule cells. Second, we found elevated hippocampal level and its mRNA abundance in aged rats (24-28 months) compared with 6 month old rats. The increase in the expression of dynorphin was correlated with the decreased release of glutamate in the hippocampus. Taken together, these data clearly indicate that glutamate plays a major role in regulating the expression of dynorphin.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90057-02 LMIN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of Epileptiform Activity by Various Excitatory Amino Acid Inhibitors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Clifford L. Mitchell Pharmacologist LMIN NIEHS

Others:	J. S. Hong	Pharmacologist	LMIN	NIEHS
	C. W. Xie	Visiting Fellow	LMIN	NIEHS
	P. Lee	Visiting Associate	LMIN	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Neurophysiology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

Considerable work in this laboratory has focused on the role of excitatory amino acids in seizure activity and related sequelae. The major objective of this project is to determine the relative contributions of the kainate, quisqualate and NMDA receptors in the generation of epileptiform activity along the tri-synaptic pathway of the hippocampal formation. Sustained stimulation of the perforant path activates this entire pathway. The NMDA antagonist, MK-801, prevents status epilepticus and loss of the CA1 and CA3 pyramidal cells associated with this stimulation. However, paroxysmal shaking of the head, neck and trunk, a phenomenon known as wet dog shaking (WDS) is exacerbated by MK-801. Since WDS are associated with epileptiform activity of the dentate granule cells these results suggest that NMDA receptors may be of little importance in the generation of epileptiform activity at the perforant path - dentate granule cell synapse. However, NMDA receptors appear to be critical for establishment of status epilepticus and subsequent death of the CA1 and CA3 pyramidal cells. On the other hand, we have found that gamma-D-glutamylglycine ( $\gamma$ -DGG) a non-specific glutamate receptor antagonist, elevates the threshold for eliciting WDS. Since the NMDA receptor antagonist MK-801 does not have this effect, it appears that kainic acid and/or quisqualate receptors are most important at the perforant path - dentate granule cell synapse for generation of WDS. Current studies are examining this hypothesis by examining the effect of a specific kainic acid - quisqualate receptor blocker on the threshold for eliciting WDS and epileptiform activity generated by perforant path stimulation.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90058-02 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Role of Excitotoxins on Brain-Endocrine Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Negro-Vilar	Chief	LMIN	NIEHS
Others:	F. Lopez	Visiting Scientist	LMIN	NIEHS
	T. Ionue	Visiting Fellow	LMIN	NIEHS
	I. Merchenthaler	Visiting Scientist	LMIN	NIEHS
	A. O. Donoso	Guest Researcher	LMIN	NIEHS

COOPERATING UNITS (if any)

Laboratorio de Investigaciones Cerebrales, CONICET, Mendoza, Argentina

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Excitatory amino acids constitute a novel class of neurotransmitters, glutamate and aspartate being the most representative transmitters of the family. These substances can also induce extensive neuronal injuries, and can therefore act as excitotoxins. In addition to the toxic effects of these agents, they also exist in physiological conditions in the nervous system and, therefore, may contribute to the control of physiological events. Recently, we characterized the pharmacological profile of glutamate receptors in the hypothalamus in terms of the ability of glutamate to induce LHRH release. We established a dose-response relationship between glutamate and LHRH release from arcuate nucleus-median eminence terminals in vitro. In these studies, we demonstrated that glutamate-induced LHRH release was mainly mediated through kainate-quisqualate receptor type. These observations led us to postulate that excitatory amino acid neurotransmission may be an important input in regulating LHRH release. Studies from other laboratories have indicated that endogenous excitatory amino acid administration or the blockade of endogenous amino acid neurotransmission may alter the onset of puberty in both monkeys and rats. Prompted by our observation of the importance of kainate-quisqualate receptors in the control of LHRH secretion and also by the availability of specific EAA receptor blockers, we initiated a series of studies in order to characterize the role of endogenous amino acids in the control of some physiological events. In an initial study, we observed that both a competitive NMDA receptor antagonist, AP7, and a competitive non-NMDA receptor antagonist, DNQX, were able to block, when administered into the third ventricle, the estradiol-induced LH surge in ovariectomized female rats. This provided evidence for a role of endogenous excitatory amino acids in a well characterized classic endocrine model. These and ongoing studies on the spontaneously occurring proestrous LH surge should help to establish a role for the endogenous excitatory neurotransmitters in reproductive physiology.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90059-01 LMIN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Pathways Involved in Receptor Regulation of Neurotransmitter Levels

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: M. McMillian Senior Staff Fellow LMIN NIEHS

Others: J.S. Hong Pharmacologist LMIN NIEHS

P.M. Hudson Biologist LMIN NIEHS

D. Hu Stay-in-Schooler LMIN NIEHS

R. Tuominen Visiting Associate LMIN NIEHS

H. Ye Guest Worker LMIN NIEHS

H.H. Suh IRTA Fellow LMIN NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Neuropharmacology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.3

## PROFESSIONAL:

2.8

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The purpose of this project is to determine second messenger pathways involved in receptor regulation of neurotransmitter levels. Adrenal medullary cells were used as a model neuronal system to study calcium-mobilizing receptors and their effects on protein kinase C and short- and long-term enkephalin and catecholamine secretion. Changes in intracellular calcium were studied using Fura2,  $^{45}\text{Ca}^{2+}$  influx was used to study the contribution of extracellular calcium, and [ $^3\text{H}$ ]phorbol dibutyrate binding was used to follow protein kinase C translocation. Nicotine and membrane depolarization produced a pronounced increase in intracellular calcium and short-term secretion, and was surprisingly effective in stimulating PKC translocation. Extracellular calcium was required for these effects. In contrast, angiotensin II and other phospholipase C-linked receptor agonists produced a transient increase in intracellular calcium regardless of extracellular calcium concentration. Single cell studies suggest that the apparent transient response reflects oscillations above and below the basal calcium level, which continues for at least an hour. These oscillations are dependent on extracellular calcium as is short-term secretion. Angiotensin II stimulates a conotoxin-sensitive calcium influx which may be distinct from the angiotensin effect on phospholipase C. The relative roles of calcium influx, intracellular calcium mobilization and protein kinase C activation in long-term secretion and gene regulation are presently being studied. Previous work from this lab has demonstrated that splanchnic innervation exerts a predominantly inhibitory effect on enkephalin and catecholamine gene transinjection; inhibitory effects on  $\text{Ca}_i$  responses can be seen under some conditions with GABA, histamine and ATP. Possible mechanisms of action of these inhibitory agonists are being examined. In addition to providing further insight into the mechanisms of action of different receptor agonists, these studies should lead to a better understanding of how second messenger signals are integrated for long term effects.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90060-01 LMIN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotransmitter Secretion and Gene Expression Regulated by Angiotensin System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Harold H. Suh ERTA Fellow LMIN NIEHS

Others:	J. S. Hong	Pharmacologist	LMIN	NEIHS
	M. McMillian	Senior Staff Fellow	LMIN	NIEHS
	E. Mar	IPA	LMIN	NIEHS
	P. M. Hudson	Biologist	LMIN	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Neuropharmacology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.2

## PROFESSIONAL:

2.0

## OTHER:

0.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The long-term goal of the proposed study is to understand the long-term effect of angiotensin II and the endogenous renin-angiotensin system (ERAS) in relation to the mechanism of regulation of the neurotransmitters release and the gene expression of adrenal cell proteins (proenkephalin, tyrosine hydroxylase, and PNMT). The specific goal of this project was to determine if the release of the neurotransmitters (catecholamines and met-enkephalin) and gene expression by long-term exposure to angiotensin II (AgII) is mediated by protein kinase C (PKC) or arachidonic acid (AA) and its metabolite (eg. PGE<sub>2</sub>). We have used primary cultured bovine adrenal chromaffin cells. We have found that AgII, AA and PGE<sub>2</sub> increased the secretion of met-enkephalin (ME) after continuous stimulation for 12 hrs. The secretion of ME by AgII, AA and PGE<sub>2</sub> was completely inhibited by staurosporin but not by K252a, indicating that PKC plays an important role in mediating the secretion of ME. The action of staurosporin seems to be selective since staurosporin did not affect the secretion induced by potassium and veratridine. We also found that the secretion of ME induced by AgII and AA was not inhibited by indomethacin (a cyclooxygenase inhibitor) and caffeic acid (a lipoxygenase inhibitor), indicating that secretion of ME induced by AgII and AA is not mediated by PGE<sub>2</sub>. Currently, we are trying to determine if the secretion of ME induced by AgII is mediated by AA, using PLA<sub>2</sub> inhibitor. We are also studying the effects of staurosporin, K252a, indomethacin and caffeic acid on the gene expression of proenkephalin induced by AgII, AA or PGE<sub>2</sub>. This study will delineate the mechanism underlying the regulation of adrenal medullary function of sympathetic neurotransmission. This work should provide rational means for intervening in cardiovascular diseases in which blood pressure and heart rate may be modified by AgII.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		<b>PROJECT NUMBER</b> Z01 ES 90061-01 LMIN
<b>PERIOD COVERED</b> October 1, 1989 to September 30, 1990		
<b>TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)</b> Molecular Mechanisms of Gene Regulation in Adrenal Chromaffin Cells		
<b>PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)</b>		
PI:	Eng-Chun Mar	IPA LMIN NIEHS
Others:	J. S. Hong W. Q. Zhang Harold Suh P. M. Hudson	Pharmacologist Visiting Associate Visiting Fellow Biologist LMIN NIEHS LMIN NIEHS LMIN NIEHS LMIN NIEHS
<b>COOPERATING UNITS (if any)</b>		
<b>LAB/BRANCH</b> Laboratory of Molecular and Integrative Neuroscience		
<b>SECTION</b> Neuropharmacology Section		
<b>INSTITUTE AND LOCATION</b> NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2	0.2
<b>CHECK APPROPRIATE BOX(ES)</b> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
<b>SUMMARY OF WORK (Use standard unabbreviated type. Do not exceed the space provided.)</b> <p>           The major goal of this study is to understand the mechanism(s) underlying the activation of catecholamine enzymes [Tyrosine Hydroxylase (TH), Phenylethyl-N-methyltransferase (PNMT)] and opioid neuropeptide [Proenkephalin (PENK)] gene expression in response to a variety of effector stimuli in bovine adrenal medullary chromaffin (BAM) cells in vitro. When BAM cells were exposed to nicotine and TPA separately or together, the secretion of Met-ENK, the processed products of preproenkephalin was increased. A bimodal Met-ENK release mechanism was observed. For short-term effect the increase of Met-ENK secretion in modulator treated BAM cells was independent of new protein synthesis. On the other hand, the long-term effect of Met-ENK release required new protein synthesis because the presence of cycloheximide, a protein synthesis inhibitor effectively blocked the Met-ENK exocytosis. We observed at least an additive effect of Met-ENK secretion by nicotine and TPA together in BAM cell culture for short-term and long-term release. Furthermore, the PENK transcription was also elevated in drug-treated cells, indicating a transcription-secretion coupling mechanism in these studies. Based on the known genomic upstream sequence from the transcription site of human and rat ENK, we first used the AP-1 consensus responsive element to study the AP-1 related transcription factor(s) involved in the activation of ENK expression. The data of our DNA-protein interaction and gel shift studies demonstrated a good correlation between the elevation of PENK transcription and increase of the AP-1 transactivator. In summary, we have observed a transcription and secretion coupling effect of Met-ENK by nicotine-, TPA-, and nicotine and TPA-treated BAM cells. The new protein synthesis was only required for the long-term effect of secretion. An additive effect was seen with Nicotine in combination with TPA in BAM cell culture. Furthermore, at least a transcription factor, namely AP-1 protein was also stimulated in drug-treated cells, implying that the AP-1 trans-activating factor might be involved in PENK transcription activation.         </p>		





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90062-01 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hypothalamic Control of Pulsatile Prolactin Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Francisco J. Lopez Visiting Scientist LMIN NIEHS

Others: Andres Negro-Vilar Chief LMIN NIEHS  
Edwin Meade Jr. Biological Lab. Technician LMIN NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.3

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is today well recognized that all pituitary hormones in particular, and hormones in general, are secreted in an intermittent, pulsatile manner. This special manner of secretion appears to be of paramount importance in encoding regulatory signals; i.e. stimulatory, inhibitory, which are integrated at the target organ level to obtain a determined target organ response to the signal. Recently, our group has provided new insights concerning the characterization and the regulation of pulsatile prolactin secretion. In fact, pulsatile prolactin secretion dramatically varies depending on the estrous cycle stage in rats, suggesting that different patterns convey distinct signals to target tissues. In an attempt to decode these putative signals, we evaluated the total amount of hormone released per pulse (area under the pulse), and with the aid of frequency distribution analysis, we observed that at least two different classes of pulses could be defined; big and small mass prolactin pulses. Interestingly, the maximum incidence of big mass prolactin pulses was observed during the afternoon of estrus, an estrous cycle stage in which prolactin secretion appears to protect the corpus luteum from the luteolytic actions of LH secreted during metestrus. This observation suggested to us that, in fact, prolactin actions on the corpus luteum are encoded in these big mass prolactin pulses. In addition, big mass prolactin pulses seem to be precisely regulated by sporadic interruptions in the dopaminergic inhibitory tone exerted by the hypothalamus in both male and female rats. Initial experiments, using D2 dopaminergic receptor antagonists, showed that the dopaminergic tone appears to be required to observe big mass prolactin pulses. Experiments in which the dopaminergic tone is either completely abolished or continuously present will demonstrate whether sporadic interruptions of the dopaminergic tone are involved in the genesis of big mass prolactin pulses.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 90063-01 LMN

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Galanin in the Regulation of Gonadal Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Francisco J. Lopez Visiting Scientist LMN NIEHS

Others: Andres Negro-Vilar Chief LMN NIEHS

Edwin Meade, Jr. Biological Lab Technician LMN NIEHS

Miriam G. Wisniewski Biologist LMN NIEHS

## COOPERATING UNITS (if any)

Department of Veterinary Anatomy, Ohio State University, Columbus, Ohio

## LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

## SECTION

Reproductive Neuroendocrinology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.8

## PROFESSIONAL:

0.3

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

Galanin is a peptide originally isolated from porcine intestine and shown to be vastly distributed in the central nervous system. The recent availability of the synthetic rat molecule allowed us to generate a specific antiserum against rat galanin. In initial studies from our lab, we observed that a subset of rat galanin-immunoreactive neurons in the preoptic area of the hypothalamus presented a morphology and location similar to that of LHRH-immunoreactive neurons. In fact, these GAL-immunoreactive neurons also expressed LHRH immunoreactivity. This observation suggested to us that GAL could be an important factor in regulating gonadal functions. Using an incubation system [arcuate nucleus-median eminence (AN-ME) fragments] developed and characterized in our lab, we observed that, indeed, rat galanin was able to potentially stimulate LHRH release from AN-ME terminals in vitro. This stimulatory action was linked to PGE<sub>2</sub> release since the blockade of PG synthesis using indomethacin, a cyclooxygenase inhibitor, abolished rGAL-induced LHRH release. Moreover, rGAL-induced LHRH release requires a functional noradrenergic system to be expressed since an  $\alpha$ -adrenergic antagonist, phentolamine, as well as a specific  $\alpha_1$ -adrenergic antagonist, prazosin, were able to block rGAL-induced LHRH release. These anatomical and functional correlates, indicated to us that GAL and LHRH could be also co-secreted. Indeed, when we analyzed rGAL and LHRH release into the hypophyseal portal circulation it was observed that rGAL is released into the portal circulation in higher concentrations than those observed in peripheral blood. Furthermore, rGAL secretion into the portal blood occurred in a pulsatile fashion, depicting secretory events that coincided with those of LHRH. However, it must be noted that practically all rGAL secretory episodes preceded those of LHRH, suggesting that in the generation of a LHRH pulse GAL may be the trigger. These observations provide the basis for considering GAL as a hypothalamic factor participating in the regulation of LHRH release and, thereby, the control of gonadal functions.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 ES 90064-01 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Endocrinology of Normal and Abnormal Puberty

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Penelope K. Manasco Senior Staff Fellow LMIN NIEHS

Others: Andres Negro-Vilar Chief LMIN NIEHS  
Lillian Barrett Biological Lab Technician  
Louis Underwood Pediatric Endocrinology UNC-CH

COOPERATING UNITS (if any)

Division of Pediatric Endocrinology, Department of Pediatrics, University of North Carolina, Chapel Hill, NC

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

.9

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

In normal adults, the secretory function of the gonads is stimulated by the pituitary gonadotropins, LH and FSH. The gonadal steroids, in turn, modify secretion of LH and FSH through a feedback loop. During childhood, pituitary LH and FSH secretion is relatively low; gonadal secretions are low, and the hypothalamus, the principal regulator of the system, has a high "set-point" of responsiveness to gonadal steroids. As a result, the hypothalamic-pituitary-gonadal axis is in a relatively quiescent state. With the onset of puberty the hypothalamic "set-point" becomes more sensitive to the stimulatory and maturational effects of gonadal steroids; the production of LH and FSH increases; and in turn, the secretion of gonadal hormones increases. Secretion of the pituitary gonadotropins, LH and FSH, are modulated not only by sex steroids, but also by a gonadal protein, inhibin. Inhibin is a glycoprotein having a molecular weight of approximately 32,000 daltons in the human. It is secreted by testicular sertoli cells and ovarian granulosa cells. The literature contains only a single report of serum concentrations of inhibin during puberty. This report details the results of a cross-sectional study done on single blood samples collected from 99 boys and 102 girls of pubertal ages. We are studying how the gonadotropins, inhibin, and the sex steroids interplay during the normal pubertal process and in children with abnormal puberty by serially measuring plasma levels of these hormones over a 10 hour period and in response to stimulation with luteinizing hormone releasing hormone. In addition, we are studying how the bioactivity of the gonadotropins change in children with central precocious puberty after treatment with a long acting LHRH agonist. We hope that these studies will allow us to better understand how environmental factors such as environmental estrogens can affect the interplay of hormones during the pubertal process.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 90065-01 LMIN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Isolation of a Testis Stimulating Factor in Familial Male Precocious Puberty

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Penelope K. Manasco Senior Staff Fellow LMIN NIEHS

Others: Andres Negro-Vilar Chief LMIN NIEHS  
Lillian Barrett Biological Lab Technician LMIN NIEHS  
Barry D. Albertson Assoc. Professor Univ. Oregon  
Janet Jones Research Nurse Clinical Ctr NIH  
Louisa Laue Senior Staff Fellow DEB NICHD

COOPERATING UNITS (if any)

Division of Endocrinology, University of Oregon; Developmental Endocrine Branch, NICHD; Clinical Center, NIH

LAB/BRANCH

Laboratory of Molecular and Integrative Neuroscience

SECTION

Reproductive Neuroendocrinology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

.7

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The mechanism for the onset of puberty in Familial Male Precocious Puberty (FMPP) has never been understood. It is a gonadotropin independent form of puberty and no bioactive substance could be identified in the plasma of these boys using a rat testicular model. We developed a unique bioassay model using intact cynomolgus monkeys that had been pretreated with a LHRH antagonist to remove their endogenous gonadotropins. In this model, we were able to demonstrate that infusion of plasma from a boy with FMPP into the testicular artery caused a significantly greater testosterone response than infusion of plasma from a prepubertal control. Now that we have demonstrated the presence of this factor, we developed an in vitro system to isolate the factor. The system which shows the greatest promise is a mouse Leydig cell tumor line which responds to cAMP but not to gonadotropins. When plasma from boys with FMPP is placed in the media, there is greater hormone plasma from production than when plasma from prepubertal boys is placed in the media. Normal adult males have an intermediate response. At present, we are refining the techniques and continuing to collect plasma from boys with FMPP being studied by the investigators in DEB/NICHD.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25020-08 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Pulmonary Surfactant System and its Modification by Toxic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	G. E. R. Hook	Research Chemist	LPP, NIEHS
Others:	W. E. Bakewell	Graduate Student	LPP, NIEHS
	C. J. Viviano	Graduate Student	LPP, NIEHS
	R. Kumar	Visiting Fellow	LPP, NIEHS
	S. Hill	Technician	LPP, NIEHS
	D. Noel	Technician	LPP, NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Pulmonary Pathobiology

## SECTION

Biochemical Pathology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.75

## PROFESSIONAL:

2.75

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pulmonary surfactant is a heterogeneous complex consisting of surface active phospholipids and specific proteins synthesized and secreted by Type II cells in the alveoli of the lungs. The major lipid component of surfactant is dipalmitoylphosphatidylcholine and the major protein component is surfactant protein A (SPA). SPA is a specific surfactant-associated protein found only in the lungs. The function of DPPC is to lower surface tension at the air/cell interface and the function of SPA appears to be to assist in this process. Biosynthesis and secretion of these substances by alveolar Type II cells is critical for the stabilization and function of the lungs.

Silica dust causes massive increases in the pulmonary content of both surfactant phospholipids and proteins. The induction of surfactant production in the lungs appears to be a result of the silica-induced inflammatory condition and the mechanism through which this occurs is currently under investigation. Some, but not all, Type II cells within the alveoli become hypertrophic and we have shown that these type II cells are in an activated state with regards to their ability to synthesize both surfactant phospholipids and SP-A. These hypertrophic Type II cells are responsible for the massive increases in surfactant found in the lungs of silica-exposed rats. At the present time we are trying to determine the mechanism through which intratracheal injection of silica leads to activation of alveolar type II cells.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25021-07 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Regulation of Differentiation of Tracheobronchial Epithelial Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A.M. Jetten	Senior Staff Fellow	LPP, NIEHS
Others:	E.E. Floyd	Staff Fellow	LPP, NIEHS
	T. Vollberg	Staff Fellow	LPP, NIEHS
	C. Nervi	Visiting Fellow	LPP, NIEHS
	N. Saunders	Visiting Fellow	LPP, NIEHS
	M. George	Chemist	LPP, NIEHS
	K. Marvin	IRTA Fellow	LPP, NIEHS
	S. Bernacki	Guest Worker	LPP, NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Pulmonary Pathobiology

## SECTION

Cell Biology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

5

## PROFESSIONAL:

4

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies have been focussing on the mechanisms that regulate the proliferation and differentiation in normal tracheobronchial and epidermal epithelial cells. For this purpose human bronchial and epidermal, and rabbit tracheal epithelial cells are used as in vitro model systems. A multi-stage program of hyperplasia and squamous metaplasia has been proposed. Transforming growth factor  $\beta$  and retinoids influence the proliferation and differentiation of these cells. TGF- $\beta$ 1 and TGF- $\beta$ 2 inhibit cell growth but do not induce squamous differentiation. TGF- $\beta$  treatment induces several gene products including transglutaminase type II, fibronectin, collagen IV and laminin. This increase is observed at the protein as well as the mRNA level. The regulation of some of these genes is dependent on protein synthesis and the state of differentiation of the tracheobronchial epithelial cell. Several cDNA clones were isolated that encode mRNA's abundantly expressed in squamous differentiated cells and present at low abundance in undifferentiated cells. These cDNA's have been sequenced and amino acid sequence deduced from their respective DNA sequence. The clone SQ37 represents a 1.0 kb mRNA which encodes a proline-rich protein containing a tandem repeat of eight amino acids. pTG-7 represents a 3.6 kb mRNA encoding transglutaminase type I. Retinoic acid suppresses the expression of SQ37, transglutaminase type I, involucrin and several other squamous cell-specific mRNA's. Tracheobronchial and epidermal epithelial cells contain nuclear retinoic acid receptor (RAR) activity. These cells express relatively high levels of RAR $\alpha$  and RAR $\gamma$  and low levels of RAR $\beta$ . Retinoic acid treatment increases the level of RAR $\beta$  mRNA in tracheobronchial but not in epidermal cells. We propose that these RAR's mediate the action of retinoids on gene expression in tracheobronchial epithelial cells. Lung carcinoma cells show an aberrant expression of RAR $\beta$  and RAR $\gamma$ . A defective expression of RAR's may play a role in establishing a malignant phenotype in these cells.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25023-07 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Mechanisms of Progression of Transformed RTE Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Paul Nettesheim	Chief	LPP, NIEHS
Others:	A. Robertson	Senior Staff Fellow	LPP, NIEHS
	R. Steigerwalt	Senior Staff Fellow	LPP, NIEHS
	P. Ferriola	Staff Fellow	LPP, NIEHS
	T. Gray	Biologist	LPP, NIEHS
	V. Godfrey	Biologist	LPP, NIEHS
	D. Rusnak	Technician	LPP, NIEHS
	S-Y. Zhu	Visiting Associate	LPP, NIEHS
COOPERATING UNITS (if any)	Wundhaug	IRTA Fellow	LPP, NIEHS

## LAB/BRANCH

Laboratory of Pulmonary Pathobiology

## SECTION

Epithelial Carcinogenesis Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

8

## PROFESSIONAL:

6

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are exploring the role of growth factors in the proliferative control of normal and transformed airway epithelial cells. An in vitro model of rat tracheal epithelial cell (RTE) neoplastic progression is being used to examine TGF $\alpha$  and TGF $\beta$  signalling pathways. TGF $\alpha$  binds to the EGF receptor and is mitogenic while TGF $\beta$  binds to unique receptors and inhibits the growth of many cells, including those of the tracheo-bronchial tract. Normal RTE cells in culture are highly growth factor dependent and require EGF, while transformed cell variants are EGF independent. Both normal and transformed cells express TGF $\alpha$  mRNA transcripts, but expression is significantly higher in most neoplastic cell lines. TGF $\alpha$  is detected in media conditioned by transformed cells by RIA and Western blotting and addition of TGF $\alpha$  antibodies to the media inhibits growth. Tyrphostin, an EGF receptor tyrosine kinase inhibitor also inhibits growth of transformed cells, presumably by blocking ligand induced mitogenic signals. These results indicate that TGF $\alpha$  acts as an autocrine growth regulator of transformed RTE cells.

Normal and transformed RTE cells also express TGF $\beta$  transcripts, however, transformed cells secrete significantly less of this negative growth factor. Preliminary evidence suggests that transformed cells are less competent in activating latent TGF $\beta$  than normal cells. TGF $\beta$  responsiveness appears to be highly regulated in normal RTE cells since cells in late log and plateau phases of growth are far less TGF $\beta$  responsive than cells in early growth phase. In contrast, several transformed cell lines are either completely unresponsive or markedly hypo-responsive to TGF $\beta$  growth inhibition, regardless of growth state. Whether this difference between normal and transformed RTE cells is due to altered TGF $\beta$  processing, TGF $\beta$  binding or post-receptor signalling is under investigation.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25027-07 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

**Identification and Characterization of Materials Secreted by Pulmonary Clara Cells**

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. E. R. Hook Research Chemist LPP, NIEHS

Others: D. Dixon Expert CPB, NIEHS

## COOPERATING UNITS (if any)

Cell Biology Group (A. M. Jetten)  
Epithelial Carcinogenesis Group (P. Nettesheim)

## LAB/BRANCH

Laboratory of Pulmonary Pathobiology

## SECTION

Biochemical Pathology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The functions of the bronchiolar Clara cell are not known although it is generally believed that the cell is secretory. Using Clara cells isolated from the lungs of rabbits we have shown that the major protein secreted by bronchiolar Clara cells is a low molecular weight protein immunochemically and compositionally similar to uteroglobin, the major secretory protein of the uterine epithelium. Rabbit tracheal cells also secrete uteroglobin although morphologically there are no cells in the tracheal epithelium identical to bronchiolar Clara cells. We have identified tracheal epithelial cells containing uteroglobin by using an immunogold procedure. These investigations reveal that uteroglobin-containing tracheal cells, like those of the bronchioles are nonciliated and contain abundant smooth endoplasmic reticulum. However, unlike the bronchiolar Clara cell, the tracheal cell is tall and columnar in overall shape and it contains few secretory granules.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25030-04 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Cellular and Biochemical Mechanisms of Particle-Induced Lung Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Arnold R. Brody	Research Biologist	LPP, NIEHS
Others:	V. Kalter	Staff Fellow	LPP, NIEHS
	A. Osornio	Visiting Fellow	LPP, NIEHS
	J. Bonner	Staff Fellow	LPP, NIEHS
	L. Moore	Biologist	LPP, NIEHS
	A. Badgett	Biologist	LPP, NIEHS
	W. Ussler	Postdoctoral	LPP, NIEHS
	P. Coin	Guest Worker	LPP, NIEHS
COOPERATING	R. Schapira	Guest Worker	LPP, NIEHS
	T. Purdue	IRTA Fellow	LPP, NIEHS

## LAB/BRANCH

Laboratory of Pulmonary of Pathobiology

## SECTION

Pulmonary Pathology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

7

## PROFESSIONAL:

5

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research in the pulmonary pathology laboratory has been focused upon the basic biological mechanisms through which inhaled particles cause lung disease. We have developed models of asbestosis and silicosis using rats and mice and have shown that the disease process is initiated at junctions of bronchioles and alveolar ducts. One hour of exposure to chrysotile asbestos is sufficient to cause progressive fibrogenesis at alveolar duct bifurcations. The process is initiated by a complement-dependent chemoattraction of lung macrophages to the sites of particle deposition. The central working hypothesis in our laboratory is that these macrophages synthesize and secrete an array of products which mediate the pathogenesis of lung fibrosis. Studies over the past years have shown that macrophages produce a wide array of arachidonic acid metabolites. These are potent inducers of inflammation and cell migration. Now we are focusing upon a group of cell-derived proteins collectively termed "growth factors". The growth factor which is the most potent inducer of mesenchymal cell (e.g. fibroblasts and smooth muscle cells) proliferation is platelet-derived growth factor (PDGF). The most potent stimulator of extracellular matrix production by mesenchymal cells is transforming growth factor beta (TGF $\beta$ ). We have shown that lung macrophages secrete generous quantities of both of these factors. Thus, we postulate that inhalation of toxic particles activates populations of lung macrophages which produce PDGF and TGF $\beta$ , the factors which could mediate the consequent fibrogenic response. To test this hypothesis, an extended series of experiments is ongoing to understand the biology, biochemistry and molecular nature of the growth factors in asbestos exposed animals and their cells. Most recently we have shown that alpha-macroglobulin ( $\alpha$ -M), a high molecular weight antiprotease, is secreted by lung macrophages and serves as a specific binding protein for the macrophage-derived PDGF. Then we showed that the  $\alpha$ -M enhances, by more than 100%, the ability of PDGF to stimulate the proliferation of rat lung fibroblasts. Efforts to block the biological activity of the growth factors with appropriate antibodies in vivo have just been initiated.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01ES 25032-01 LPP

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Regulation of Proliferation and Differentiation in Airway Epithelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Paul Nettesheim	Chief	LPP, NIEHS
Others:	S. Randell	Staff Fellow	LPP, NIEHS
	T. Shimizu	Visiting Associate	LPP, NIEHS
	L. Kaartinen	Guest Researcher	LPP, NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Laboratory of Pulmonary Pathobiology

## SECTION

Epithelial Carcinogenesis Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

8

## PROFESSIONAL:

6

## OTHER:

2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The tracheo-bronchial tree is lined by an epithelium composed of three major cell types: ciliated, secretory and basal cells. The purposes of our studies are: 1) to define tracheo-bronchial subpopulations in terms of critical biochemical markers; 2) To determine the pathway of differentiation in various segments of the conducting airways and 3) To identify major regulatory systems controlling growth and differentiation in this organ system. Previous research in the field of airway cell differentiation has been severely limited by dependence upon morphological criteria to define the different cell types and by the lack of an in vitro cell culture system that fully supports muco-ciliary differentiation. We have sought to identify cell type-specific biochemical markers. Cytochemical studies showed that basal cells express cell surface  $\alpha$ -linked terminal galactose residues. Highly purified, viable basal cell and nonbasal cell preparations were isolated using lectin reactivity and flow cytometry. Twelve monoclonal antibodies against rat tracheal epithelial cells were produced; all stained subsets of tracheal cells including 3 which are specific for secretory cells and one which is specific for ciliated cells. Using immunocytochemistry, keratin profiles of different cell types were examined. Keratin 14 was found to be an excellent basal cell specific marker and keratins 5, 8, 18 and 19 were expressed to a much greater degree in non-basal cells. We established an air-liquid interface culture system that supports muco-ciliary differentiation. We identified culture media components essential for mucous cell differentiation. In recent experiment the attachment substratum was modified such that ciliated cell differentiation also occurs. When used in conjunction with the cell type-specific markers described above, this in vitro system will enable us to perform investigations of factors important for the regulation of proliferation and differentiation of tracheo-bronchial cells. Our current work is focused on TGF $\alpha$ , TGF $\beta$ , retinoids, extracellular matrix molecules and their receptors.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70060-17 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Biology/Toxicology of Estrogenic Environmental Chemicals

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. A. McLachlan	Head, Develop. Endocrinol. and Pharmacol.	LRDT NIEHS
Others:	R. R. Newbold	Biologist	LRDT NIEHS
	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
	C. T. Teng	Senior Staff Fellow	LRDT NIEHS
	N. Bossert	Staff Fellow	LRDT NIEHS
	C. Burroughs	Staff Fellow	LRDT NIEHS
	D. Ignar	PRAT Fellow	LRDT NIEHS
	C. Hebert	Visiting Fellow	LRDT NIEHS

## COOPERATING UNITS (if any)

Bowman-Gray School of Medicine	University of North Carolina
Duke University Medical Center	University of Würzburg

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Developmental Endocrinology and Pharmacology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

9.8

## PROFESSIONAL:

5.8

## OTHER:

4.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies have continued to determine the molecular and cellular targets of estrogenic chemicals and establish the mechanisms by which interactions of estrogens with developing genital tract target cells result in permanently altered differentiation, including dysmorphology and neoplasia. In the developmentally-estrogenized mouse, neoplasias in the female structures derived from the Müllerian duct (e.g., uterus) were demonstrated to be hormonally dependent, transplantable tumors; cell lines established from them were shown to progressively lose the estrogen receptor (ER) as well as hormone-dependent growth. The chronological expression of oncogenes and tumor suppressor genes is also being studied. The developing Müllerian duct has been further studied at the cellular and molecular levels. It was determined that in the immature mouse uterus, the epithelium was relatively deficient in detectable ER although the mitogenic signal of estrogen is apparently transduced in ER deficient epithelial cells. The presence of epidermal growth factor receptor (EGFR) in these cells suggests that the mitogenic signal of estrogens early in development may involve growth factors such as EGF. This is further supported by experiments in immature mice in which EGF can replace estrogen in stimulating uterine and vaginal growth and differentiation. Studies on phospholipid turnover (especially phosphoinositides) may provide further insights into signal transduction mechanisms associated with estrogens. The growth characteristics of murine uterine epithelium and stroma were compared in serum-free tissue culture. The relative role of tissue-specific factors versus blood-borne cellular factors was examined in the newborn mouse uterus using the enzyme peroxidase as a marker for eosinophilic responses. These studies together support a complex developmental progression in hormone responsiveness in the female genital tract with important clues for neoplasia.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70062-03 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Growth Factors in Growth and Differentiation of the Reproductive Tract

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. G. Nelson	Senior Staff Fellow	LRDT NIEHS
Others:	Y. Sakai	Visiting Scientist	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Developmental Endocrinology and Pharmacology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.5

## PROFESSIONAL:

2.0

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our goal is to understand the cellular mechanism by which prenatal and neonatal DES exposure permanently alters the cell growth and differentiation of male and female reproductive tracts. Current research involves the elucidation of the role of peptide growth factors (IGF-1, EGF, TGF $\alpha$ , TGF $\beta$ <sub>1</sub>, TGF $\beta$ <sub>2</sub>, and lactoferrin) in the regulation of reproductive function and steroid hormone action. Our studies have now provided considerable evidence that EGF-like peptides (EGF and TGF $\alpha$ ) and the EGF-receptor play crucial roles in mediating estrogen action in the adult female mouse reproductive tract. In addition, the growth of the undifferentiated neonatal mouse reproductive tract (a stage of development that is exquisitely sensitive to DES-induced carcinogenesis) is also regulated in part by EGF and TGF $\alpha$ . Interestingly, the EGF receptor is ontogenically a much earlier constituent of the epithelium of the developing uterus than is the estrogen receptor which appears relatively late in development. These data suggest that EGF/TGF $\alpha$  are intimately associated with the normal development and functioning of the reproductive tract and may play a role in DES-induced carcinogenesis and teratogenesis. Recent *in vivo* experiments clearly demonstrate that EGF has a significant effect on the male reproductive tract. Also, *in vivo* studies have identified TGF- $\beta$ <sub>1</sub> and - $\beta$ <sub>2</sub> as potential regulators of uterine and vaginal growth. Lactoferrin, an estrogen-inducible iron-binding protein, may play a critical role in the growth and differentiation of the reproductive tract and mammary gland. At present, there are still many important questions regarding the role of growth factors on reproduction. Our future plans are to continue to characterize and define the role of peptide mediators of estrogen-induced growth, determine the cell type responsible for the synthesis of these factors, locate the cellular target where these factors act, elucidate whether these factors act alone, synergistically, or temporally, and investigate the second messenger systems that transduce the growth factor signal.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70065-14 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical-Receptor Interactions in Reproduction and Hormonal Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. S. Korach	Head, Receptor Biology	LRDT NIEHS
Others:	K. Chae	Research Chemist	LRDT NIEHS
	V. Davis	IRTA Fellow	LRDT NIEHS
	J. A. McLachlan	Head, Develop. Endocrinol. and Pharmacol.	LRDT NIEHS
	M. Ikeda	Visiting Associate	LRDT NIEHS
	S. Migliaccio	Visiting Associate	LRDT NIEHS
	H. Kohno	Visiting Fellow	LRDT NIEHS
	W. Beckman	Expert	LRDT NIEHS

## COOPERATING UNITS (If any)

University of Würzburg	Burroughs Wellcome Research Labs
Laboratory of Molecular Biophysics, NIEHS	UNC Medical School
Medical Foundation of Buffalo	Duke University Medical School

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Receptor Biology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

10.2

## PROFESSIONAL:

6.8

## OTHER:

3.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Induction of certain genes by estrogens involves the interaction of the hormone with a receptor protein. The specificity and responsiveness of tissues to hormonal stimulation are governed in most part by the presence and biochemical action of this receptor protein. We have purified and characterized the receptor protein and its intracellular site(s) of action. Earlier observations had indicated during uterine estrogen stimulation a bimodal nuclear receptor occupancy. New findings have shown a change in chromatin receptor acceptor sites and nuclear matrix binding coordinately with the receptor pattern indicating a possible alteration in the pattern of gene expression at the different times. The estrogen receptor protein has been purified from mouse uterus by steroid affinity and oligonucleotide chromatography. Molecular properties of the protein have been analyzed by epitope specific antibodies to understand the mechanism of receptor activation and conformation. Characterization of the receptor has indicated multiple forms which are proteolytic fragments and not separate gene products. The protease action results in a receptor form which has lost its ability to interact with other transcription factors and DNA responsive sequences and, consequently, its biological activity. We have shown that the nuclear estrogen receptor specifically exhibits a doublet form when bound by biologically active estrogens. Studies of receptor DNA interactions have indicated multiple complexes by band shift assays. The specificity and stability of these complexes varies depending on the biological potency of the ligands. The higher molecular weight component of the doublet is phosphorylated and associated with tightly bound chromatin sites. Weak estrogens or antiestrogens transiently produce the doublet form. These findings suggest that this form of the estrogen receptor may be involved in gene activation and hormone responsiveness. Cell culture studies have indicated the production of stable transfectant clones of the estrogen receptor and reporter gene constructs. These systems are being used as in vitro test systems for studies of estrogen receptor protein structure and gene regulation.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70067-07 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanism of Steroid Hormone in Sex Organ Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. T. Teng	Senior Staff Fellow	LRDT NIEHS
Others:	Y. H. Liu	Visiting Fellow	LRDT NIEHS
	J. A. McLachlan	Head, Developmental Endocrinology and Pharmacology	LRDT NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Developmental Endocrinology and Pharmacology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.7

## PROFESSIONAL:

2.0

## OTHER:

1.7

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lactoferrin (LF) is synthesized and secreted by the uterine epithelial cells during proestrus and estrus. There is no detectable lactoferrin protein in uterine epithelium during metestrus and diestrus. A high level of lactoferrin was detected by both immunostaining and *in situ* hybridization in uterine epithelial cells at day 1 through day 3 of pregnancy, but disappeared completely at day 4 of pregnancy. Genomic clones containing LF gene sequences were isolated from the 129/J mouse genomic library. We found that the LF gene is organized similar to the human transferrin gene with 17 exons separated by 16 introns. Primer extension analysis demonstrated a prominent transcription initiation site at 32 bp 5' to the translation start codon. The promoter region contains two SP1 sites (-914 to -908, -535 to -529) and one AP2 site (-515 to -508). A CAAT box and a non-canonical TATA box were located at -72 and -32 respectively. An estrogen responsive element consensus sequence is located at -353 to -327. The putative ERE in mLF gene shows high homology to the Xenopus vitellogenin 2A ERE with a mutation at 3' half of the palindrome (G to A). Gel mobility shift assay, using nuclear protein extract (NPE) prepared from control and DES-treated immature mouse uterus and lung, indicates that specific protein-DNA complexes are formed with the DNA fragment containing the putative ERE. A synthetic oligonucleotide vitellogenin 2A ERE competes with the mLF ERE region for NPE effectively. DNase I footprinting with estrogen-stimulated immature mouse uterine NPE indicates that the putative mLF ERE region are protected. The past year, this laboratory has also isolated another estrogen-stimulated mouse uterine secretory protein, 63 kDa. Polyclonal antibody against 63 kDa protein was raised in rabbit. We are in the process of determining its N-terminal amino acid and partial sequencing. Human lactoferrin cDNAs from both mammary gland and bone marrow were isolated. Full length cDNA from mammary gland library was sequenced. We found one deletion near the end of the coding sequences which caused a frameshift at the 3' end of the protein.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70076-06 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Germ Cell-Specific Molecules of Spermatozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. M. Eddy	Head, Gamete Biology	LRDT NIEHS
Others:	J. E. Welch	Staff Fellow	LRDT NIEHS
	R. S. McGee	IRTA Fellow	LRDT NIEHS
	D. A. O'Brien	IPA	LRDT NIEHS

## COOPERATING UNITS (if any)

U. of Washington School of Medicine	Columbia University, College of Physicians
U. of North Carolina, Chapel Hill	and Surgeons
The Biomembrane Institute, Seattle	

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Gamete Biology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

5.3

## PROFESSIONAL

3.0

## OTHER:

2.3

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Spermatogenesis proceeds from proliferation of spermatogonial stem cells, through the unique process of meiosis in spermatocytes, to end with the dramatic morphogenesis of haploid spermatids into spermatozoa. This project examines gene products responsible for the unique structural and functional features of spermatozoa, with the goal of determining the intrinsic and extrinsic mechanisms which regulate expression of genes at different phases of the developmental process of spermatogenesis. For example, the fibrous sheath is a cytoskeletal component of the sperm flagellum assembled late in spermatogenesis. A 78kd fibrous sheath protein is synthesized during meiosis, while a 67kd protein appears in spermatids. These proteins share biochemical and functional properties with intermediate filament proteins. Monoclonal antibody and cDNA sequencing studies suggest these proteins are products of unique intermediate filament genes. Also being studied is a gene for a glyceraldehyde 3-phosphate dehydrogenase enzyme (G3PD) of spermatogenic cells. Northern blot data indicate that this gene is expressed only in spermatogenic cells, with the transcript being present in low amounts in spermatocytes and abundant in spermatids. Although not required by spermatids, this G3PD appears to accommodate unique metabolic needs of spermatozoa. These and other recent studies indicate that spermatogenic cell-specific proteins are often variants of known somatic cell proteins and the products of genes expressed only in germ cells. This suggests that unique structural and functional features of spermatozoa are the result of germ cell-specific gene expression.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 70077-04 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Expression of Heat-Shock Genes in Mouse Spermatogenic Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. M. Eddy Head, Gamete Biology LRDT NIEHS

Others: M. O. Rosario Senior Staff Fellow LRDT NIEHS

## COOPERATING UNITS (if any)

Division of Toxicology Research and Testing, NIEHS  
The University of North Carolina, Chapel Hill

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Gamete Biology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.7

## PROFESSIONAL:

1.5

## OTHER:

1.2

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The synthesis of heat-shock proteins (hsp) following environmental stress is a highly conserved response and apparently protects cells against long-term effects of adverse conditions. One of the most abundant proteins (P70) in spermatogenic cells is related closely to hsp70, as shown by ATP affinity chromatography purification, immunoblots of two-dimensional protein gels, and peptide mapping. The P70 protein is synthesized during the meiotic phase of spermatogenesis. Although several members of the hsp70 gene family have been sequenced and their mRNA expression characterized, little is known about the proteins encoded by these genes. We developed antisera specific to P70 and hsp70 to investigate the expression and role of these proteins in male germ cells. The antiserum to P70 was also used to select clones from a mouse spermatocyte cDNA library. One of these clones hybridized to a testis-specific, developmentally regulated 3.0 kb mRNA whose expression was not affected by heat. Sequence analysis indicated that the clone has high homology to previously reported genes of the hsp70 family. However, it appears that spermatogenic cells do not produce hsp70 mRNA or protein in response to heat stress. This suggests that P70 gene expression is linked to suppression of the hsp70 gene in spermatogenic cells. However, the process of spermatogenesis is unusually sensitive to elevation of temperature and to many toxic agents and P70 may function in germ cell differentiation rather than to protect against the effects of environmental stress. Although P70 expression may be beneficial to the process of spermatogenesis, this process may also leave male germ cells particularly susceptible to environmental stress.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 80040-07 LRDT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Pharmacogenetics of Microsomal Steroid Hydroxylases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Negishi	Head, Pharmacogenetics	LRDT NIEHS
Others:	K. Aida	Visiting Scientist	LRDT NIEHS
	M. Iwasaki	Visiting Associate	LRDT NIEHS
	R. Lindberg	Visiting Fellow	LRDT NIEHS
	H. Yoshioka	Visiting Fellow	LRDT NIEHS
	B. Burkhart	Biologist	LRDT NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Reproductive and Developmental Toxicology

## SECTION

Pharmacogenetics

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

8.6

## PROFESSIONAL:

6.0

## OTHER:

2.6

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects  
☐ (a1) Minors  
☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We characterized the structural genes of sex-specific mouse P450s: the male-specific steroid 16 $\alpha$ -hydroxylase (P450<sub>16 $\alpha$</sub> ) and the female-specific steroid 15 $\alpha$ -hydroxylase (P450<sub>15 $\alpha$</sub> ). The nucleotide and deduced amino acid sequences indicate that these sex-specific steroid hydroxylases have evolved as the new members within the different P450 subfamilies. Our site-directed mutagenesis studies show that P450<sub>15 $\alpha$</sub> -dependent 15 $\alpha$ -hydroxylase activity critically depends on the residue at position 209. Moreover, the cytochrome's spin equilibrium is altered by the amino acid substitutions at this position in P450<sub>15 $\alpha$</sub> , indicating that the 209 resides close to the 6th ligand of the heme protein. We confirmed, by nuclear run-on assay and the transgenic mouse, that the sex-specific P450 gene expressions were regulated by growth hormone transcriptionally. On the other hand, the P450 induction by exogenous chemicals and metals, including pyrazole and CoCl<sub>2</sub>, are regulated post-transcriptionally, but pre-translationally. We used *in vitro* transcription, DNase 1 footprinting, and gel retardation to identify two proximal cis-acting transcription elements (SDI and CTE) in the 5'-flanking region of P450<sub>16 $\alpha$</sub>  gene. Moreover, SDI is the strong one and specific for this gene and, therefore, may be involved in the male-specific gene transcription. Also, our transient transfections showed the presence of positive and negative regulatory elements (PRE and NRE) in the far upstream of the P450<sub>16 $\alpha$</sub>  gene. In addition, preliminary results suggested that these are growth hormone-response elements. It appeared, therefore, that PRE, NRE and SDI together provide P450<sub>16 $\alpha$</sub>  gene with the hormone-dependent, male-specific transcription. These results are extremely important to understand the sex- and tissue-dependent toxicity and carcinogenicity of endogenous hormones and exogenous chemicals and also their polymorphism.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 43010-05 DBRA

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Macromolecular Modeling and Carcinogenesis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	David G. Hoel	Director	DBRA/OD	NIEHS
Others:	Marshall W. Anderson	Research Chemist	DBRA/LMT	NIEHS
	Lee G. Pedersen	Research Chemist	DBRA/OD	NIEHS
	Charles Foley	Staff Fellow	DBRA/LMT	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Division of Biometry and Risk Assessment

## SECTION

Office of the Division Director

## INSTITUTE AND LOCATION

NIH, NIEHS, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.0

## PROFESSIONAL:

2.0

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with determining theoretical factors involved in mutagenesis and in the initial steps of carcinogenesis. The proliferation of new experimental techniques in genetic engineering is providing innovative pathways for studying the dependence of chemically induced mutational events on DNA sequence. Computer modeling is being used to examine the physical chemical factors (charge distributions, binding energies, stereo-chemistry, strand distortion, activation energies, solvation, counterions) contributing to site specificity of DNA damage by chemical agents. The same techniques are also being employed to determine changes in molecular properties of oncogene proteins as a consequence of specific mutations.

Specifically, computer intensive quantum mechanical calculations are employed to determine the properties of small molecules. These results are then used to parameterize empirical force fields that can in turn be used to model the mechanical properties of large molecules such as meaningful segments of DNA and proteins with molecular mechanics/dynamics and computer graphics. Software development/modifications are effected to model our large molecular systems.

Research issues of ongoing interest include the characterization of local structures of DNA sequences (native and chemically modified) that contain known mutational hotspots from mammalian oncogenes and bacterial systems, the examination of the molecular details of the initial attack by mutational metabolites, sequence dependent DNA bending, DNA-protein interactions, and the understanding of the consequences of single amino acid changes on the function of critical proteins such as the p21 ras oncogene protein. In the latter case, a new collaboration with a West German group has provided starting X-ray coordinates.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-43002-14 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Exposure to Halogenated Aromatic Compounds

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Walter J. Rogan	Chief	EB	NIEHS
Others:	Beth C. Gladen	Statistician	SBB	NIEHS
	Mei-Lin Yu	IRTA Fellow	EB	NIEHS
	Christopher J. Portier	Statistician	SBB	NIEHS

## COOPERATING UNITS (if any)

Statistics and Biomathematics Branch, NIEHS

## LAB/BRANCH

Epidemiology Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

2.3

## PROFESSIONAL

1.3

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☒ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polychlorinated biphenyls (PCBs) and the DDT/DDE (DDE is the stored metabolite of DDT) family are toxic, widespread pollutants. Both pass from mother to child through the placenta and by contaminating breast milk. This project includes a study of subjects exposed to low levels of both compounds in North Carolina, a study in Mexico where levels of DDE are two to five times higher, and a study of children poisoned in utero by PCBs in Taiwan. In North Carolina, we reported that children who were exposed to the upper 5-10 percent of background PCB levels while in utero still had detectable motor delay at 18 and 24 months. We are following the children to see if delay persists. Using data from this project, we showed that the estimated cancer risk from chemicals in breast milk is mostly from PCBs, and that estimated excess mortality from this exposure is comparable to the estimated decrease in mortality from breast feeding. We have completed field work on a study in Mexico in which we look at lactation failure in women with high levels of DDE in milk, an observation we made previously in the US. In Taiwan, an epidemic of 2000 cases of PCB poisoning occurred in 1979. In 1985, we did a survey of 117 children who were born to mothers who were poisoned. We found developmental delay and physical abnormalities did not occur in the same children, and that children with more symptomatic mothers tended to have greater delay as follow-up progressed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-43004-12 EB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Environmental Exposures and Chronic Renal and Other Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Dale P. Sandler	Epidemiologist	EB	NIHS
Others:	Walter J. Rogan	Chief	EB	NIHS
	Elizabeth A. Whelan	IRTA	EB	NIHS

## COOPERATING UNITS (if any)

Bowman Gray School of Medicine/Baptist Hospital, Duke University Medical Center, University of North Carolina Medical School, Charlotte Memorial Hospital, University of Utah College of Nursing

## LAB/BRANCH

Epidemiology Branch

## SECTION

Environmental and Molecular Epidemiology Section

## INSTITUTE AND LOCATION

NIHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.25

## PROFESSIONAL:

0.5

## OTHER:

0.75

## CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The Branch studies the role of the environment in the etiology of certain chronic diseases. Environmental exposures may increase the risk of chronic disease, either directly, or through their interaction with individual characteristics such as hormonal status or genetic susceptibility.

Data analysis continues in a multi-center case-control study of risk factors for chronic renal disease. Renal disease risk was increased for regular users of non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), especially among older men and other high risk groups. Exposure to solvents and to silica was found to increase the risk of glomerulonephritis. Regular consumption of cola beverages was associated with an overall increase in risk. This was apparently not due to caffeine or artificial sweeteners, but to other unidentified constituents or factors associated with cola consumption. Although there was no independent risk from caffeine, heavy caffeine consumers appeared to be at increased risk of nephrotoxicity from NSAIDs. Consumption of alcoholic beverages was associated with a slight increase in risk, but no risk was conferred by smoking.

To better understand the influence of hormone status on disease risk, a cohort of 1000 women who have contributed prospective menstrual and reproductive data since 1935 are being followed to determine their risk of several diseases. To date, almost 90% of the cohort has been traced, with approximately 700 found to be alive, and 200 known to be deceased. Questionnaires were sent and death certificates and medical records will be retrieved.

Previous work in the Branch has shown that x-ray evidence of asbestos exposure identifies non-occupationally exposed persons, and can predict lung cancer risk. To estimate trends in asbestos exposure, approximately 1000 x-rays from the



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-44003-13 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidemiologic Study of Reproductive Outcomes and Environmental Exposures

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Allen J. Wilcox	Chief of Section	EB	NIEHS
Others:	Donna D. Baird	Senior Staff Fellow	EB	NIEHS
	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
	Irva Hertz-Picciotto	Epidemiologist	EB	NIEHS
	Jack A. Taylor	Medical Officer	EB	NIEHS
	Paige P. Hornsby	Health Scientist	EB	NIEHS

## COOPERATING UNITS (if any)

Statistics and Biomathematics Branch, NIEHS, Laboratory of Reproductive & Developmental Toxicology, NIEHS, Columbia University, California State Health Department, Free University of Brussels, Belgium

## LAB/BRANCH

Epidemiology Branch

## SECTION

Reproductive Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

2.10

## PROFESSIONAL:

1.60

## OTHER:

0.50

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the reproductive epidemiology project is to develop and apply methods for measuring damage to human reproduction. Such damage can include infertility, endocrine dysfunction, menstrual disorders, subclinical pregnancy loss, clinically recognized pregnancy loss (miscarriage), impaired fetal growth or other intrauterine damage, and perinatal death. Many of these reproductive endpoints have been poorly studied in humans. We are exploring all these endpoints as possible tools for detecting toxic effects on human health. In several instances, we are using exposure to a known toxin as a way to develop our methods for studying reproductive effects. DES is a synthetic estrogen with well-known toxic effects in animal models. Among a group of persons exposed to DES prenatally, we are looking for effects on endocrine and menstrual function, male fertility, immune function, and cognitive development. Evidence for DES effects on any of these endpoints would justify further study of those endpoints in other populations that may be exposed to reproductive toxins. Meanwhile, we continue our development of epidemiologic methods for studying early pregnancy loss and birthweight. In addition, we are exploring genetic conditions which may affect susceptibility of populations to reproductive toxins. Specifically, we are investigating a mutation of the estrogen receptor gene which has been reported to be associated with a high risk of pregnancy loss.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-46002-06 EB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Environmental Exposures and Cancer Risk

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory, and institute affiliation)

PI: Dale P. Sandler Epidemiologist EB NIEHS

## COOPERATING UNITS (if any)

Cancer and Leukemia Group B member institutions, Roswell Park Memorial Institute,

## LAB/BRANCH

Epidemiology Branch

## SECTION

Environmental and Molecular Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS.

0.35

## PROFESSIONAL

0.35

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data collection has been completed for a case-control study of risk factors for the acute leukemias in adults in which patients can be classified according to clonal chromosome characteristics, immunologic phenotype, and other biochemical markers, as well as according to more widely available classification systems, to determine if risk factors differ for distinct subgroups of patients. The study was motivated by reports that 50 % of leukemia patients have chromosome abnormalities in bone marrow, and that these patients are likely to have had prior chemotherapy or occupational exposure to solvents. Approximately 650 patients (85% of those eligible) who were enrolled in cancer treatment protocols sponsored by Cancer and Leukemia group B, a cooperative cancer study group, were interviewed regarding exposure to solvents and chemicals, smoking, irradiation, use of potentially toxic medications, and family medical history. A total of 636 interviews were also completed with population controls, representing an 80% response rate. Data are currently being readied for analysis. Preliminary results suggest associations between cigarette smoking, marijuana use, and exposure to certain drugs and chemicals and leukemia risk.

In a related ongoing study, nearly 250 additional patients with acute myelocytic leukemia who are being studied by Cancer and Leukemia Group B for the presence of specific oncogenes have also been interviewed. Among the first 50 patients studied, a potential association has been found between Ras mutation and both cigarette smoke exposure and use of insecticides and pesticides.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-47001-04 EB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Exposure to Radon and Cancer Risk

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Dale P. Sandler	Epidemiologist	EB	NIEHS
Others: Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
Gwen W. Collman	Senior Staff Fellow	EB	NIEHS

## COOPERATING UNITS (if any)

Yale University, New Haven, Connecticut, University of Utah, Salt Lake City, Utah, and Statistics and Biomathematics Branch, NIEHS

## LAB/BRANCH

Epidemiology Branch

## SECTION

Environmental and Molecular Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.5

## PROFESSIONAL

0.5

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent surveys indicate that between 10 and 20 percent of homes in the United States have indoor radon levels that exceed EPA's guideline level for remedial action, resulting in an estimated 5,000 to 20,000 lung cancer deaths each year. This estimate is based on extrapolating from results of studies of miners with very high radon exposures; findings from these studies may not be generalizable to the population at large.

The relationship between cumulative residential exposure to radon and cancer risk is being evaluated in a collaborative study involving Yale University and the University of Utah. The study will include 1000 smokers with lung cancer, 750 nonsmokers with lung cancer, and over 2100 population controls from Connecticut, Utah, and Southern Idaho. Because smoking may enhance the effects of radon exposure, the study is designed to evaluate the potential interaction between radon and cigarette smoke exposure. Detailed residential and exposure histories are obtained through the use of computer assisted telephone interviews (CATI). Radon measurements are made in past homes using year-long alpha track etch detectors, in order to estimate cumulative radon exposure since age 25 for each subject. Complete lifetime exposure assessments (including childhood) will be made for a subset of participants. A companion study in Connecticut will evaluate the potential childhood cancer risk associated with residential radon exposure. Cumulative radon exposure will be determined for approximately 125 childhood cancer cases and 250 healthy comparison subjects. Standardized data collection instruments have been developed, and case and control identification has begun. Preliminary plans for modeling radon exposures have been formulated.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-47002-04 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Effects of Plant Estrogens in Postmenopausal Women

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Donna D. Baird Staff Fellow EB NIEHS

Others: Allen J. Wilcox Medical Officer EB NIEHS  
Clarice R. Weinberg Mathematical Statistician SBB NIEHS  
John McLachlan Chief LRDT NIEHS

## COOPERATING UNITS (if any)

Laboratory of Reproductive and Development Toxicology; Statistics and Biomathematics Branch, NIEHS

## LAB/BRANCH

Epidemiology Branch

## SECTION

Reproductive Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.55

## PROFESSIONAL:

0.55

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Exposure to highly estrogenic substances can disrupt reproduction and increase cancer risk, as well as influence bone metabolism and cardiovascular health. Chemicals that are weakly estrogenic are widespread in the environment, including several pesticides. Health effects of these environmental estrogens are not known. This project is designed to determine whether a test-case exposure to environmental estrogens, i.e., exposure to dietary soybeans (rich in plant estrogens) has the expected estrogenic effects. The experimental study compares women who were randomized to a soy-diet group with women who ate normally. We are looking for changes in LH, FSH, sex hormone binding globulin, apolipoprotein A-I, and vaginal smear cytology.

If plant estrogens are found to be biologically active in postmenopausal women, other questions to be addressed include: (1) What effects do these chemicals have on other segments of the population, especially premenopausal women and babies on soy formula? (2) Can plant estrogens promote carcinogenicity or, as suggested by some laboratory studies, do they have anticarcinogenic effects at least in subsets of the population, such as premenopausal women, who have high levels of endogenous estrogens? (3) Do effects of plant estrogens explain some of the differences in morbidity and mortality seen in vegetarians compared with nonvegetarians? (4) Can dietary changes be used in prevention or treatment of estrogen-related conditions?



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-49001-02 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Studies of Occupational Populations Exposed to Carcinogenic Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Eric S. Johnson	Visiting Scientist	EB	NIEHS
Others:	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
	George W. Lucier	Chief	LBRA	NIEHS
	Beth C. Gladen	Statistician	SBB	NIEHS

COOPERATING UNITS (if any) Laboratory of Biochemical Risk Analysis, NIEHS; Statistics and Biomathematics, NIEHS; Poultry Science Department, North Carolina State University; Regional Poultry Research Laboratories, USDA, East Lansing, Michigan; Infectious Disease Unit, Duke Medical Center

## LAB/BRANCH

Epidemiology Branch

## SECTION

Environmental and Molecular Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.15

## PROFESSIONAL:

1.15

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Two cohort mortality studies of workers exposed to oncogenic viruses in the chickens industry are being conducted, to examine if poultry workers are at increased risk of developing cancer. The study populations consist 26,000 poultry workers from Missouri and Chicago and 12,000 controls. Ascertainment of vital status has begun on the Missouri cohort, and records have been received and are being processed for the Chicago cohort. We have collected blood from 50 former poultry workers and controls, to test for antibodies to chicken oncogenic viruses. DNA is currently being abstracted from white blood cells from these samples, and will be tested for presence of integrated viral genome using the PCR technique. These studies will investigate whether humans are infected with these viruses. In vitro testing of the infectivity of reticuloendotheliosis virus for human cells is almost completed. Blood from 40 sprayers of phenoxy herbicides, and 40 controls has been collected from individuals in Australia, for the determination of serum levels of dioxins and furans, to see if persons who use these herbicides are significantly exposed to these compounds.

Analysis of data from a case-control study of lung cancer (occurring in excess) in the meat industry is being completed. It is hoped to identify the exposure(s) within the industry responsible for the excess.

Urine specimens from 45 workers in supermarkets in Albuquerque, New Mexico have been collected and are being analyzed for muconic acid, a metabolite of benzene. The purpose of this study is to see if the very small amounts of benzene emitted from the thermal decomposition of polyvinyl chloride plastic during the wrapping of meat, can be detected in the urine.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-49002-02 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Epidemiologic Studies of Cancer Susceptibility and Oncogene Activation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Jack A. Taylor	Medical Officer	EB NIEHS
Others:	Marshall W. Anderson	Chief	LMT NIEHS
	Rachel Patterson	Biologist	LMT NIEHS
	Teddy Devereux	Biologist	LMT NIEHS

## COOPERATING UNITS (if any)

Laboratory of Molecular Toxicology, NIEHS, University of North Carolina, Duke University, Roswell Park Memorial Institute, University of Georgia, Fox Chase, Telemark Sentralsjukhus (Norway), The Finsen Institute (Denmark), St. Mary's Hospital (Grand Junction, CO)

## LAB/BRANCH

Epidemiology Branch

## SECTION

Environmental and Molecular Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

1.5

## PROFESSIONAL:

1.0

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

<input checked="" type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input checked="" type="checkbox"/> (a2) Interviews		

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in the branch have been established to investigate the role of proto-oncogene alleles in cancer susceptibility, and the role of oncogenes in carcinogen-induced human tumors. To investigate the role of oncogenes in chemical carcinogenesis, fixed tissue blocks have been obtained from approximately 50 cases of benzidine or beta-naphthylamine associated bladder cancer, and 100 bladder cancer cases without such exposures. In addition, a small number of cyclophosphamide associated bladder tumors have been obtained. The polymerase chain reaction (PCR) is being used to amplify H- K- and N-ras genes followed by oligonucleotide probing or direct sequencing for oncogene activating mutations at codons 12, 13, and 61. The pattern and mutational spectra of oncogene activation will be compared between benzidine/beta-naphthylamine associated tumors, cyclophosphamide associated tumors and those which arose spontaneously or were smoking-associated.

In a similar study, fixed tissue samples of lung tumors have been obtained from individuals with primary lung cancers who had high dose occupational exposure to one of a variety of known lung carcinogens, including radon, asbestos, nickel, chromate, and vinyl chloride. PCR with oligonucleotide probing or direct sequencing is being used to characterize K-ras family mutations which will then be correlated with exposure information.

A case control study of bladder cancer has been initiated to investigate whether restriction fragment length polymorphisms of proto-oncogenes correlate with cancer susceptibility. Exposure information, along with blood, urine, and tumor tissue, are being collected on 200 bladder cancer cases and 200 controls. Southern blots are being used to determine whether rare alleles of H-ras and other proto-oncogenes correlate with cancer susceptibility. The interaction between genotype and exposure will also be explored.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-49003-01 EB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Effects on Fertility

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Donna D. Baird	Staff Fellow	EB	NIEHS
Others:	Allen J. Wilcox	Medical Officer	EB	NIEHS
	Clarice R. Weinberg	Mathematical Statistician	SBB	NIEHS
	Andrew S. Rowland	IRTA Fellow	EB	NIEHS

## COOPERATING UNITS (if any)

Statistics and Biomathematics Branch, NIEHS

## LAB/BRANCH

Epidemiology Branch

## SECTION

Reproductive Epidemiology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

1.80

## PROFESSIONAL:

0.80

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☒ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary purpose of the project on fertility is to develop methods for identifying reproductive hazards. The majority of reproductive failure in women occurs before they know they are pregnant. We are currently focussing on two ways of assessing this reproductive loss. The first is development of questionnaire methods for studying fertility. We applied this method to an occupational cohort in a study of mercury vapor and nitrous oxide exposure in dental assistants. This study also allowed us to evaluate the accuracy of mail-questionnaire data on time to pregnancy, the basic data used to compare fertility among exposed and unexposed. The second approach is focussed on developing biological markers of impaired ovarian function. Estrogen and progesterone metabolites in daily urines are being measured to characterize follicular development, ovulation, and luteal function. Ultimately, we are working toward developing methods for studying reproductive hazards in small populations so that localized exposures can be evaluated.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01-ES-21024-09 LBRA
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Drug Metabolizing Enzymes in Animal Models and Human Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI:           Joyce Goldstein                           Pharmacologist                           LBRA   NIEHS		
OTHERS:     M. Faletto                               Staff Fellow                           LBRA   NIEHS M. Romkes                              Visiting Fellow                      LBRA   NIEHS J. Blaisdell                            Biologist                             LBRA   NIEHS P. Linko                                 Chemist                               LBRA   NIEHS G. Lucier                               Chief                                  LBRA   NIEHS		
COOPERATING UNITS (if any) Judy Raucy, University of New Mexico Jack Taylor, Epidemiology Branch, NIEHS Masahiko Negishi, LRDT, NIEHS		
LAB/BRANCH Laboratory of Biochemical Risk Analysis		
SECTION Metabolism and Receptors		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 4.7	PROFESSIONAL 2.9	OTHER: 1.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>             The cytochrome P450s comprise the principle monooxygenase system which metabolizes foreign chemicals. Some of these enzymes are polymorphic. We are cloning and sequencing cDNAs for these P450s from individuals showing variations. We are then using cDNA expression systems such as yeast and COS-1 cells to assess the role of individual P450s to metabolize drugs and mutagens. We have prepared libraries from two human livers. No cDNA clones for P450IIC8 were identified in a human liver phenotypically low in IIC8 protein. Northern analysis and PCR analysis also indicate extremely low levels of the mRNA for this enzyme. However, a cDNA(254c) for a new human IIC P-450 was identified. We are searching for a full length clone to identify and express this enzyme. A second library was constructed from a human liver high in IIC8, and 90 essentially full length cDNA clones in the IIC subfamily were identified. Hybridization studies indicate that IIC8 represents 32% of the clones, and ~60% are identical or similar to IIC9 (either MP4 or MP8). Two variant full-length IIC9 clones were identified. Two additional cDNA clones representing variants of a new full-length IIC P-450(s) have been sequenced. Additional cDNAs are being characterized. Expression studies in yeast have compared expression of rat liver P450IIC13 and its phenotypic variant. P450IIC13 was expressed in yeast cells at a level 5-7-fold higher than its phenotypic variant containing 9 base substitutions, demonstrating that point mutations in the mRNA result in the defective expression of P450IIC13. We are presently using cDNA expression systems to express members of the human P450IIC subfamily. The ability of these human P450s to metabolize important drugs and environmental chemicals (mephenytoin, tolbutamide, and various classes of promutagens) will be analyzed. Liver tissue from approximately 25-50 humans will be analyzed for possible phenotypic variability of P450 enzymes using Northern analysis. These studies will assess the role of individual P450s in metabolic activation/deactivation of chemical mutagens and carcinogens.           </p>		





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-46003-06 LBRA

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Markers for Evaluating Exposure and Biologically Effective Dose

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Claudia Thompson	Senior Staff Fellow	LBRA NIEHS
	George Lucier	Chief	LBRA NIEHS

OTHERS:	D. Bell	Staff Fellow	LBRA NIEHS
	Y. Liu	Visiting Fellow	LBRA NIEHS
	Z. McCoy	Bio. Lab. Tech.	LBRA NIEHS
	C. Miller	Biologist	LBRA NIEHS
	J. Goldring	Biologist	LBRA NIEHS

## COOPERATING UNITS (if any)

Epidemiology Branch, DBRA

## LAB/BRANCH

Laboratory of Biochemical Risk Analysis

## SECTION

Cellular Epidemiology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

4.2

## PROFESSIONAL:

1.9

## OTHER:

2.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range objective is to evaluate the use of human lymphocytes as potential markers of human exposure and/or susceptibility. The effect of activation/deactivation pathways on the formation of DNA adducts and the resulting consequences on genetic endpoints of DNA damage (assessed by various methods including gene mutation) are evaluated in human lymphocytes following *in vitro* exposure to chemicals. We have shown that human lymphocytes are polymorphic in one isozyme of glutathione S-transferase (GST) referred to as the mu form (GST-mu). Approximately 50% of the population has high GST-mu activity and 50% has low activity. A significant correlation is seen for GST-mu activity between human liver and lymphocytes suggesting that human lymphocytes are an appropriate surrogate tissue for liver GST-mu. GST's play key roles in the metabolic detoxification of PAH's whereas this enzyme activates ethylene dibromide (EDB) to a DNA reactive species. A significant correlation between DNA adducts of benzo(a)pyrene (BP) and BP metabolism was seen in human lymphocytes for persons having high GST-mu activity. In cell-free experiments, the importance of GST-mu on adduct formation by BP and aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) has been evaluated. Our results indicate that much higher concentrations of AFB<sub>1</sub>-derived adducts are formed in systems containing low GST mu activity compared to systems containing high GST-mu. These data suggest an important role for GST-mu in the detoxication of AFB<sub>1</sub>. A 10-fold variation in DNA damage, measured by nucleoid sedimentation, is seen in human lymphocyte incubated *in vitro* with EDB. Moreover, there is a strong correlation between DNA damage and the level of EDB-DNA binding. We have verified by HPLC that the primary adduct produced by lymphocytes is the S-[2-N<sup>7</sup>-guanyl]-ethyl]glutathione and it is responsible for the DNA damage observed. Species comparisons of lymphocytes responses to EDB shows that the order of sensitivity is humans > rats > mice and this species comparison is correlated with GST activity. Current studies are evaluating the relationship between polymorphisms in drug-metabolizing enzymes, DNA adducts and mutation spectra of the hprt gene.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-46004-06 LBRA

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Receptor Interactions for TCDD and Its Structural Analogs: Species Comparisons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George Lucier Chief LBRA NIEHS  
Joyce Goldstein Pharmacologist LBRA NIEHS

OTHERS: F-H. Lin IRTA Fellow LBRA NIEHS  
G. Clark IRTA Fellow LBRA NIEHS

COOPERATING UNITS (if any)

Chemical Pathology Branch, NIEHS; Statistics and Biomathematics Branch, NIEHS  
Systemic Toxicology Branch, NIEHS  
Chemical Industries Institute for Toxicology

LAB/BRANCH

Laboratory of Biochemical Risk Analysis

SECTION

Metabolism and Receptors

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are enormous species differences in the acute toxicity for TCDD and its structural analogs such as the polychlorinated dibenzofurans (PCDFs). These compounds appear to exert their effects in in vivo and in vitro systems through a mechanism requiring the Ah receptor. TCDD is a potent hepatocarcinogen in female rats but not male rats. Our studies focused on potential mechanisms for the observed sex specificity by evaluating histological and biochemical parameters in a two-stage model for hepatocarcinogenesis in female rats using diethylnitrosamine (DEN) as the initiating agent and TCDD as the promoting agent. Increases in preneoplastic foci were detected in intact rats and to a lesser extent in ovariectomized rats. This finding was consistent with the cell proliferation data which demonstrated that TCDD markedly increased the labelling index of hepatocytes only in intact rats. These data suggest that ovarian hormones (probably estrogens) play a significant role in the hepatocarcinogenic actions of TCDD. Dose-response relationships for effects of TCDD on receptor pathways important to regulation of cell division are being evaluated in the rat tumor promotion model. Results revealed that effects on epidermal growth factor receptor and estrogen receptor correlated with tumor promotion but induction of cytochrome P-450 isozymes did not. In order to address the issue of human sensitivity to the effects of TCDD and PCDFs, we have examined placentas from humans exposed to PCDFs in Taiwan and compared biochemical changes in human placenta to those occurring in rats. Our data reveal that humans are a sensitive species to PCDFs based on enzyme induction and effects on EGFR. Current studies are using rat, mouse and human lymphocyte samples to better characterize species differences in response to TCDD exposure. Other studies are investigating the mechanistic bases for interindividual variation in responsiveness to TCDD.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-48005-03 LBRA

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Mechanisms Related to Risk Factors of Mammary Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Richard DiAugustine Research Chemist LBRA NIEHS

OTHERS: S. Snedeker Senior Staff Fellow LBRA NIEHS  
 G. Jahnke IRTA Fellow LBRA NIEHS  
 G. Lucier Chief LBRA NIEHS  
 M. Walker Chemist LBRA NIEHS  
 C. Brown Biologist LBRA NIEHS

## COOPERATING UNITS (if any)

Epidemiology Branch, DBRA  
 University of North Carolina, Chapel Hill, NC  
 University of South Carolina, Charleston, SC

## LAB/BRANCH

Laboratory of Biochemical Risk Analysis

## SECTION

Hormones and Cancer

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.8

## PROFESSIONAL

2.3

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Approximately one out of every ten females born today in the U.S. will develop breast cancer. This high incidence has prompted the need to understand the biochemical basis for susceptibility to this disease. Ovarian steroids are known to have an essential role in this disease but it is not understood how these compounds function in stimulating growth of either the normal gland or breast carcinoma. In one study just recently completed, we investigated estrogen-dependent mammary gland development (ductal morphogenesis) for local expression of members of the epidermal growth factor (EGF) family. Transcripts of EGF and TGF- $\alpha$  (transforming growth factor- $\alpha$ ) were identified and immunolocalization studies revealed that these factors are made in separate cell populations of the gland. Both polypeptides were able to stimulate growth of the "regressed" gland of ovariectomized mice in vivo. These findings suggest that estrogen-stimulated ductal growth is mediated by a local EGF-like polypeptide and that synthesis of the EGF-receptor is not apparently dependent directly on ovarian steroids. Inhibitors of the EGF-receptor pathway are currently being evaluated for inhibition of ductal growth in vivo. We are also investigating the functional relationships between ductal epithelial cells in adult animals that contain EGF and similar ductal cells that contain estrogen and progesterone receptors. Comparisons will be made between the rodent gland and human breast tissue obtained by reduction mammaplasty. A class of proteases (kallikreins) that might be important in the processing of the EGF precursor (~140 kD) has been detected in the lactating mammary gland which synthesizes the EGF precursor. These enzymes are currently being evaluated for their capacity to be hormonally regulated and also for their role in converting the membrane-bound precursor to the active EGF peptide. In another study, we are investigating murine mammary preneoplastic lesions for ectopic expression of prolactin and related proteins that might account for the immortalization and phenotypic properties of these neoplasms.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01-ES-70069-08 LBRA
PERIOD COVERED <u>October 1, 1989 to September 30, 1990</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>DNA Adducts in Human Lymphocytes and Hormone-Dependent Cancers</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Richard DiAugustine      Research Chemist George Lucier              Chief	LBRA    NIEHS LBRA    NIEHS
OTHERS:	C. Thompson              Senior Staff Fellow G. Jahnke                  IRTA Fellow M. Walker                  Chemist	LBRA    NIEHS LBRA    NIEHS LBRA    NIEHS
COOPERATING UNITS (if any) Epidemiology Branch, DBRA Hormonal Carcinogenesis Laboratory, Washington State University		
LAB/BRANCH Laboratory of Biochemical Risk Analysis		
SECTION Hormones and Cancer		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             A wide variety of chemical compounds are capable of causing tumor formation in mammals. Some of these compounds become genotoxic by forming covalent modifications of DNA either directly or through reactive metabolites. The formation of these DNA adducts is considered to be a common mechanism by which structurally diverse chemicals ultimately produce mutations and cancer. The <sup>32</sup>P-postlabeling method is a sensitive technique whereby lipophilic or bulky adducts, such as those derived by exposure to polycyclic aromatic hydrocarbons can be detected in preparations of DNA. We have previously shown that multiple adducts are visualized on thin-layer maps when human lymphocyte DNA is analyzed by the <sup>32</sup>P-postlabeling method. Using the P<sub>1</sub>- nuclease-modification of this method, we found a range of one adduct per 10<sup>7</sup>-10<sup>8</sup> nucleotides for the subjects studied and that each individual had a unique adduct profile. During the latest period covered, we focused on refining the method with regard to improving sensitivity and chromatographic consistency. The modifications are being applied in two separate studies. In one investigation, we are evaluating whether pregnancy/lactation provides a permanent influence on the capacity of the mouse mammary gland to repair DNA adducts produced by treatment with benzo[a] pyrene. In another study, we are evaluating <sup>32</sup>P-postlabeling thin-layer maps of kidney DNA obtained from hamsters treated with various estrogens known to induce tumor formation in this organ.           </p>		





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-35005-11 LMT

## PERIOD COVERED

October 1, 1990 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Carcinogens-Induced DNA Damage and Cell Transformation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Marshall Anderson	Research Chemist	LMT	NIEHS
	Steven Belinsky	Senior Staff Fellow	LMT	NIEHS
	Fred Tyson	Senior Staff Fellow	LMT	NIEHS
	Theodora Devereux	Research Biologist	LMT	NIEHS
Others:	Catherine White	Biologist	LMT	NIEHS
	Molly Vallant	Biologist	LMT	NIEHS

## COOPERATING UNITS (if any)

Dr. Maronpot, Chemical Pathology Branch, NIEHS

## LAB/BRANCH

Laboratory of Molecular Toxicology

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

5.5

## PROFESSIONAL:

3.5

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The major focus of these studies is to identify critical target genes and alterations in biochemical pathways which are involved in cell transformation. Some of the biological endpoints being examined include DNA adduct formation, gene expression, and oncogene activation. Activation of the K-ras proto-oncogene appears to be one step in the development of mouse lung tumors. In order to identify other factors involved in tumor progression, epithelial cell lines are being developed from these murine tumors. The expression of growth factors, proto-oncogenes, and proteases are being characterized in these cell lines as well as solid tumors. Preliminary data indicates that gene expression in these murine tumors is similar to that observed in human lung tumors. Subtractive cDNA cloning will also be employed using cell lines or benign and malignant tumors to identify specific proteins whose expression or suppression may be involved in the progression from benign to malignant to a fully metastatic phenotype. Although activation of the K-ras gene has been associated with the induction of lung tumors in the A/J and C3H mouse following treatment with 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), this gene is not activated in lung tumors induced in the rat by this carcinogen. The nude mouse tumorigenicity assay is being employed to attempt the detection of novel transforming genes in NNK-induced rat lung tumors. The progression of pulmonary neoplasia has been characterized in the A/J mouse. The role of the type II cell as a progenitor for pulmonary neoplasia is suggested by ultrastructural findings and supported further by the fact that an activated K-ras oncogene is detected in approximately 90% of hyperplasias and contains the same activating mutation present in the carcinomas induced in this mouse strain by NNK. Since a significant number of human pulmonary cancers are thought to arise from type II cells and 30 to 40% of these neoplasms contain an activated K-ras gene, the identification of metabolic factors involved in cell transformation in this murine model could further our understanding of the development of human lung cancer.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-46005-06 LMT

## PERIOD COVERED

October 1, 1990 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oncogene Activation in Rodent and Human Tumors

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steve Reynolds	Expert	LMT	NIEHS
	Marshall Anderson	Chief	LMT	NIEHS
	Colleen Anna	Biologist	LMT	NIEHS
	Rachel Patterson	Microbiologist	LMT	NIEHS
	Greg Solomon	Biologist	LMT	NIEHS
	Katie Brown	Biologist	LMT	NIEHS
	Jonathan Wiest	IRTA Postdoctoral	LMT	NIEHS

## COOPERATING UNITS (if any)

Dr. Maronpot, National Toxicology Program, NIEHS

## LAB/BRANCH

Laboratory of Molecular Toxicology

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

6.5

## PROFESSIONAL:

2.5

## OTHER:

4.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have investigated proto-oncogene activation in chemical-induced and spontaneous rodent tumors as well as some types of human tumors. Induction of tumors in rodents by genotoxic carcinogens results in activation of specific oncogenes with high frequency. For example the activation of K-ras oncogene is one important mutational event in the development of pulmonary adenocarcinoma in both human and rodent tumors. Activated K-ras genes have been detected at a high frequency in both spontaneously occurring and chemical induced lung tumors from rodents. Chemical induced tumors examined include those derived from treatment with 1,3-butadiene, tetranitromethane, methylene chloride, benzo(a)pyrene, urethane and NNK (a tobacco specific nitrosamine). Activated K-ras genes have been detected in 40% of human pulmonary adenocarcinomas. In addition, N-ras, H-ras and two uncharacterized oncogenes have been detected in both adenocarcinomas and squamous cell carcinomas by the nude mouse tumorigenicity assay; 12 of 14 human lung tumors examined tested positive. Clones of fragments of the unknown genes have been isolated and considerable progress has been made in sequencing these fragments. In addition an activated H-ras detected in a squamous cell carcinoma does not contain activated lesions in the coding region of the H-ras gene. The data suggests that the oncogene lesion resides in the regulatory region of the gene and attempts are underway to find it. A novel type of mutational activation has also been characterized in K-ras oncogene detected in a spontaneous mouse lung tumor and in two mouse liver tumors induced by furan. Thirty base pair inserts in the second exon of K-ras were observed in these tumors. These inserts lead to tandem repeats in the K-ras gene. Examination of proto-oncogene activation in mouse tumors induced by various environmental and occupational carcinogens (i.e., methylene chloride, chlordane, tetrachloroethylene, trichloroethylene) in both susceptible and resistant strains is also in progress. These approaches may enable us to more accurately estimate risk of cancer in humans exposed to specific classes of carcinogens.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 40004-13 SBB

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Statistical Methods in Epidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Clarice Weinberg Mathematical Statistician SBB NIEHS

OTHERS: Beth Gladen Statistician SBB NIEHS  
Takashi Yanagawa Visiting Fellow SBB NIEHS  
Dale Sandler Epidemiologist EB NIEHS

COOPERATING UNITS (if any)

Epidemiology Methods Section, NCI

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Statistics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.05

PROFESSIONAL:

1.05

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

This project involves the development and application of new methodologies to epidemiologic research. (1) The use of randomized recruitment in case-control studies was further developed; (2) collaborative work continued toward developing methods for handling missing data in case-control studies; (3) a generalization of the Mantel-Haenszel estimator was developed that is applicable to data from 2xJ tables; (4) a method was developed to take geographic variability into account when computing standardized mortality ratios; (5) the effects of exposure measurement errors and population mobility were assessed in relation to designing studies of residential radon exposure and risk of lung cancer; (6) the effects of outcome misclassification (false positives and false negatives) in studies of early pregnancy loss were evaluated; (7) the effect of misclassification of time to pregnancy data on study power and bias was assessed by simulation; and (8) work continued in development of an algorithm for identifying the day of ovulation based on urinary levels of ovarian steroid hormones.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 44002-14 SBB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mathematical Modeling of Molecular Phenomena

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Norman L. Kaplan Research Mathematician SBB NIEHS

OTHERS: Dennis Boos Research Mathematician SBB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Statistics and Biomathematics Branch

## SECTION

Mathematical Modeling Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.9

## PROFESSIONAL

0.0

## OTHER

0.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research has continued on developing methodology for analyzing molecular population genetics data. A statistical test was devised for determining the presence of geographic subdivision of populations that uses molecular variation in samples of DNA sequences from two or more different localities. A permutation method was used to assess the statistical significance of the test statistic. Work has begun to study the statistical properties of the sample site frequency spectrum  $F=\{f(i), 1 \leq i \leq n-1\}$  where  $f(i)$  equals the number of segregating nucleotide sites in a random sample of  $n$  genes with frequency  $i/n, 1 \leq i \leq n-1$ . For selectively neutral variation, the distribution of the sample site frequency spectrum is a mixture of multinomials and the mixing distribution depends on the genealogical history of the sample. This representation is important because it can be exploited to make inferences about the evolutionary forces responsible for the observed variation. Work is continuing on describing the effects of strongly selective substitutions on the coalescent process. Of particular interest is when two or more strongly selective mutations are simultaneously going to fixation.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 45001-10 SBB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Design and Data Analysis Methodology for Animal Experiments

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Joseph K. Haseman	Research Mathematical Statistician	SBB	NIEHS
OTHERS:	Gregg E. Dinse	Mathematical Statistician	SBB	NIEHS
	Beth Gladen	Statistician	SBB	NIEHS
	Christopher J. Portier	Mathematical Statistician	SBB	NIEHS
	Paige L. Williams	Mathematical Statistician	SBB	NIEHS

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Statistics and Biomathematics Branch

## SECTION

Statistics Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.35

## PROFESSIONAL:

2.1

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- |   |  |                                      |
|---|--|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |                                      |
| <input type="checkbox"/> (a2) Interviews    |  |                                      |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with statistical methodology issues in the design, analysis, and interpretation of laboratory animal experiments. Specific research efforts include (1) To address the false positive issue in long term rodent carcinogenicity studies, the use of statistical methodology that adjusts for multiple comparisons was investigated. An actual case study utilizing three different statistical decision rules to evaluate 25 NCI carcinogenicity studies was examined. Agreement among these decision rules was shown to be greater than originally reported. (2) Evaluation of results from the National Toxicology Program's carcinogenicity studies has resulted in the formulation and publication of an historical control tumor database and the identification of potential sources of variability in tumor rates. (3) Various experimental designs for assessing male reproductive function in rabbits were studied to determine whether they had sufficient power to detect a reasonably sized toxic effect. It was determined that studies which employed a pre-exposure period would be appropriate. (4) Statistical methods have been developed for extracting information on disease incidence from data on disease mortality. Under certain simplifying assumptions, the disease mortality rates can be expressed in terms of the incidence and lethality rates. The proposed approach is appropriate for the case in which there are two populations, one having data on disease incidence and disease lethality and the other having data on disease mortality. Based on a model linking the incidence rates in the two groups, the incidence rates in the second population can be estimated by maximizing a likelihood that involves only mortality data. Future research includes a comparison of statistical methodologies for evaluating tumor data that do not require cause of death determinations and assessing the effects of diet and caging protocols on variability in control tumor incidence.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 48001-03 SBB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Analysis of Data from Genotoxicity Experiments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Walter W. Piegorsch Mathematical Statistician SBB NIEHS

OTHERS:

Michael A. Resnick	Supervisory Research Geneticist	CGTB NIEHS
Errol Zeiger	Supervisory Microbiologist	CGTB NIEHS
Michael D. Shelby	Supervisory Geneticist	CGTB NIEHS
Jack B. Bishop	Research Geneticist	CGTB NIEHS
Jacqueline Hughes-Oliver	Mathematical Statistician	SBB NIEHS

COOPERATING UNITS (if any)

Cellular Genetics and Toxicology Branch, DTRT

LAB/BRANCH

Statistics and Biomathematics Branch

SECTION

Biomathematics Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS

1.7

PROFESSIONAL

1.3

OTHER

0.4

CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this project is the development of appropriate statistical methodologies for analysis of genotoxicity data from a variety of animal and microbial systems. Investigations continued into the statistical analysis of data on aneuploidy induction (chromosomal loss or gain) in yeast. Statistical methods were constructed using parametric models that incorporate the unique downturn in dose-response seen in aneuploidy experimentation. Application to recently-published data showed a greater sensitivity of the new methods to correctly identify aneuploidy induction than that seen with previous methods. Investigations were also begun into the modeling and analysis of data from developmental toxicity experiments, with specific attention directed at the dominant lethal assay in the male mouse. Of interest is identification of possible overdispersion induced by treating paternal units and then sampling progeny from such experiments. Statistical methods for addressing such data were considered that require minimal assumptions on the distribution of the response; these include bootstrapping and rank-based analyses.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 48002-3 SBB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Models in Toxicology and Biochemistry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Christopher J. Portier Mathematical Statistician SBB NIEHS

OTHERS:	Norman L. Kaplan	Research Mathematician	SBB	NIEHS
	Lutz Edler	Visiting Scientist	SBB	NIEHS
	Michael W. Carr	Mathematical Statistician	SBB	NIEHS
	Walter Rogan	Epidemiologist	EB	NIEHS

Department of Mathematics and Statistics, Miami University of Ohio, Oxford, Ohio  
 Department of Biostatistics, German Cancer Research Center, Heidelberg, FRG  
 Department of Biostatistics, University of North Carolina, Chapel Hill, NC  
 Department of Biostatistics, Merrell Dow Research Institute, Cincinnati, Ohio.

## LAB/BRANCH

Statistics and Biomathematics Branch

## SECTION

Risk Methodology Section

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.2

## PROFESSIONAL:

1.5

## OTHER:

0.7

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project is intended to increase our understanding of the use and application of models in toxicology and biochemistry and to implement new mathematical models to aid in explaining current research findings. The research effort explores a diverse range of biological areas including carcinogenesis, pharmacology, developmental biology and immunology. In carcinogenesis, the adequacy of the multistage model of carcinogenesis for estimating tumor incidence rates in untreated animals was evaluated. Also, an estimate of carcinogenic potency from animal experiments which adjusts for chemically related changes in survival has been developed and is being evaluated. A mathematical model of carcinogenesis which explicitly incorporates DNA repair into the multistage process is under development. The mathematical details allowing us to use the model have been developed and current research is focused on using data for implementing this model for example chemicals. In another research area, the design of animal experiments, the implications of a clonal two-stage model of carcinogenesis on the design of the two-year rodent bioassay was evaluated. A simple design using age-dependent dosing schemes produced unique patterns of tumor incidence which can provide better information on carcinogenic mechanism. This research is continuing with emphasis on the number of stages in the carcinogenesis process. We are also studying the shape of carcinogenesis dose-response curves using information on biological activity of the chemical. Choosing the proper shape (threshold or not) is critical to an accurate estimate of carcinogenic risk. In teratology, several risk assessment models were evaluated for agreement with existing data. No model was found to be adequate and some were found to produce misleading results. Another effort in teratology concerns the ability of resampling techniques and quasi-likelihood methods to account for interlitter correlations. Additional research into the estimation of risks of mortality and morbidity from exposure to immunosuppressive chemicals has begun.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21098-04 CCB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adverse Effects of Lindane in B6C3F1 Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: H.L. Hong Biologist CCB NIEHS

Others: G.A. Boorman D.V.M., Ph.D., Chief CCB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Study Conduct Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Lindane (r-Hexachlorocyclohexane) is a widely used insecticide and may be widespread at low concentrations in the human diet. The bone marrow is a very sensitive indicator of toxicity, therefore, the possible myelotoxicity of lindane was investigated. Male B6C3F1 mice were given lindane by gavage at doses of 0, 10, 20, or 40 mg/kg B.W. for three consecutive days. Bone marrow progenitor cells numbers, hematological, and histopathological evaluations were done on days 1, 2, and 14 days following the final exposure. Lindane treated or vehicle control mice were subjected to two or four biweekly sublethal whole body irradiations (200 rads). These mice were examined at one week following the second and fourth irradiation which was 36 and 64 days following the final gavage exposure, respectively. Lindane exposure alone caused a dose-dependent decrease in erythocyte precursors and macrophage-granulocyte progenitor cell numbers which returned to control values by two weeks. Prior exposure to lindane rendered the mice more susceptible to subsequent irradiation with a delayed recovery in bone marrow progenitor cell numbers. This effect was also reflected in marrow hypocellularity at the highest lindane dose following the fourth irradiation. This study shows that lindane has a myelotoxic effect in mice and that short-term lindane exposure can induce a residual progenitor cell damage that can be demonstrated by subsequent irradiation. (These results were published in Fed. Proc. part I, #2809, 1990.)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21111-03 CCB

## PERIOD COVERED

October 1, 1989 to April 30, 1990

TERMINATED April 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stability and tissue Reaction of an Implantable Identification Device

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	G. N. Rao	D.V.M., Ph.D.	CCB	NIEHS
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Others:	J. Edmondson	Biologist	CCB	NIEHS
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## COOPERATING UNITS (if any)

Comparative Medicine Branch, Division of Intramural Research

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Laboratory Animal Management

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS

0.5

## PROFESSIONAL:

0.1

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Due to the unreliability of traditional methods, it was necessary to investigate more dependable identification methods that can be read directly or by electronic means. A two-year study to determine the stability of and tissue reaction to a microchip glass-sealed device implanted in subcutaneous tissue of mice was conducted. Seventy B6C3F1 mice/sex were anesthetized and implanted with the microchip. The devices were read by an electronic detector and palpated at periodic intervals. Ten mice/sex were necropsied at 3 months and at 15 months with the remaining animals necropsied at 24 months. Of the 140 devices implanted, 3 were lost and 4 failed during the 24-month study. Devices were palpable and appeared to be fixed at one location with no obvious swelling due to inflammation or palpable masses around the implants for 24 months. At the 3, 15, and 24 month necropsies, implants were encapsulated by connective tissue. Light microscopic evaluation indicated that the capsule around the implants was thin and composed of fibrocytes and mature collagen fibers, with minimal to mild inflammation and occasional granulomatous reaction. Neoplastic changes were not observed in the tissue around the glass-sealed devices with polypropylene cap for up to 24 months.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21112-03 CCB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Growth Patterns of F344 Rats Fed NIH-07 and NTP-88 Diets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory, and institute affiliation)

PI:	G. N. Rao	D.V.M., Ph.D.	CCB	NIEHS
Others:	J. Edmondson	Biologist	CCB	NIEHS

## COOPERATING UNITS (if any)

Comparative Medicine Branch, Division of Intermural Research

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Laboratory Animal Management

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.1

## OTHER:

0.9

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The maximum mean body weights of rats attained during the course of two-year studies increased by about 20% from 1975 to 1985. Higher body weights will lead to increases in the incidences of mammary tumors, pituitary tumors, and possibly other tumors. Modification of diet and feeding procedures may slow the growth and lower the maximum body weight attained which in turn may decrease the incidences of spontaneous tumors. Lower protein diet may decrease the incidence and severity of kidney disease. The purpose of this study is to determine the feasibility of a 15% protein diet (NTP-88) with restricted feeding from 4 p.m. to 8 a.m. in lowering the maximum body weights and decreasing the severity of nephrosis of rats in comparison with Ad libitum feeding and 24% protein diet (NIH-07). Groups of 25M + 25F F344 rats housed 5/cage by sex were fed NIH-07 or NTP-88 diet Ad libitum or 4 p.m. to 8 a.m. daily. Body weights and feed consumptions were determined at one- to eight-week intervals. Water consumption and urine analysis were done at selected intervals and at the end of the 24 month study. Kidneys are being processed for microscopic evaluation of severity of kidney disease.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21113-03 CCB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Myelotoxicity in Mice Caused by Drinking Mixture of Groundwater Contaminants

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.L. Hong Biologist CCB NIEHS

Others: G.A. Boorman D.V.M., Ph.D., Chief CCB NIEHS  
R.S.H. Yang PH.D. STB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Study Conduct Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.75

## PROFESSIONAL:

0.75

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies concerning the health effects of groundwater contaminants have been focused primarily on cancer as an endpoint. In the present studies, bone marrow parameters were monitored in mice exposed to 0, 1, 5, and 10% of a chemical mixture in drinking water for 17 days or up to 32 weeks. The mixture consisted of 25 common groundwater contaminants frequently found near toxic waste dumps, as determined by EPA surveys. Mice exposed to 5 and 10% of stock solution for 15.5 weeks showed suppression of granulocyte-macrophage progenitor cells and erythroid precursors with few or no effects on body weight, histopathology and peripheral blood counts. Mice were allowed to recover for 10 weeks at which time they received whole body irradiation. Previously chemical-treated mice were more sensitive to irradiation than untreated controls. Furthermore, synergistic effects of chemical and irradiation were demonstrated by continuing chemical exposure with multiple irradiation. The effect became more pronounced following multiple irradiation and the recovery of progenitor cells occurred more slowly. Thus, chemical exposure caused a significant residual marrow damage that was not apparent with routine hematological or pathological techniques, but could be demonstrated by subsequent irradiation. These results suggest that long-term exposure to highly contaminated groundwater may present a subtle risk to the hematopoietic stem cells. (The results were published in Fed. Proc. part II, #5599, 1989, Europ. J. Pharmacol, 1990, and Tox. Lett. 49:183-197, 1989.)





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21115-03 CCB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of d-Limonene on Alpha 2U-Globulin in Rat Kidney

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.L. Hong	Biologist	CCB	NIEHS
Others:	S. Eustis	Ph.D.	CCB	NIEHS
	G.A. Boorman	D.V.M., Ph.D., Chief	CCB	NIEHS
	M. Elwell	Ph.D.	ETB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Study Conduct Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

d-Limonene is a natural component of a variety of foods and beverages and is found in many fruits, vegetables, meats, spices and other food items. Recently NTP found conducted chronic two-year studies of d-Limonene in rats and mice and found there was clear evidence of carcinogenic activity for male F344 rats only as shown by increased incidences of tubular cell hyperplasia, adenomas and adenocarcinomas of the kidney. The response observed in male rats may be linked to specific renal perturbation of alpha 2U-globulin, unique to the male rat kidney. This study was performed to evaluate the hyaline droplet formation and the presence of alpha 2U-globulin was determined by the enzyme linked immunosorbent assay (ELISA) in the kidney homogenates. Total protein were measured in the aliquots for alpha 2U-globulin by the Lowry method. We have confirmed that d-Limonene produced significant dose-related increase of alpha 2U-globulin only in male rats. These results suggest that d-Limonene associated nephrotoxicity in male rats may be related to altered catabolism of alpha 2U-globulin, a low molecular weight protein synthesized by the liver under androgenic control. Thus we developed the ELISA technique for alpha 2U-globulin and refined the procedures for our use in the future research projects at NTP and NIEHS. (These results were published in the Toxicologist, 1990.)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21125-01 CCB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pesticide and Fertilizer Mixture Study

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.L. Hong Biologist CCB NIEHS

Others: G.A. Boorman D.V.M., Ph.D., Chief CCB NIEHS  
R.S.H. Yang Ph.D. STB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Study Conduct Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL:

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human exposure to chemicals is rarely limited to one single chemical. Recent concern about groundwater contamination in agricultural areas has led the NTP to initiate a study of the toxicology of pesticide/fertilizer chemical mixtures which are based on confirmed groundwater contamination in California and Iowa. While the long-term toxicity studies were being done on contract, specific myelotoxicity and residual effects of bone marrow were investigated. Bone marrow parameters were monitored in mice exposed to 0, 1X, 10X, and 100X of chemical mixture II or III drinking water for 3 to 15 weeks. Some mice were allowed to recover for 8 weeks after cessation of chemical treatment, then received whole body irradiation 200 rads twice at 8-week intervals. Examinations of hematology and histopathology were also performed.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21126-01 CCB

## PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Evaluation of L-Tryptophan Toxicity in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.L. Hong	Biologist	CCB	NIEHS
Others:	G.A. Boorman	D.V.M., Ph.D., Chief	CCB	NIEHS
	M. Thompson	Ph.D.	ETB	NIEHS
	M. Elwell	Ph.D.	ETB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Study Conduct Section

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL

0.3

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently 875 cases (4 fatal) of eosinophilia-myalgia-syndrome (EMS) has been reported in the U.S.A. There appears to be an association with ingestion of tryptophan or certain lots of tryptophan. The purpose of this study is to determine if one or more of the several lots of L-tryptophan submitted by the Center of Disease Control (CDC) has the potential to cause toxicity in female B6C3F1 mice. Mice were given L-tryptophan in 0.5% methylcellulose-water at doses of 0 or 300mg/kg B.W. daily from Monday to Friday for 21 days over a 30-day period by gavage. Animals were killed at 3, 17 and 30 days after the first treatment. Multiple hematological and clinical pathology examinations were performed during the dosing with histopathological and hematopoietic evaluations at the terminal sacrifice. The results of this study provided no indication of toxicity in mice that could be interpreted as related to the EMS as seen in man. The B6C3F1 mouse may be not be a sensitive model or the lots tested may not contain the contaminant(s) that causes EMS in man.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21141-01 CCB

## PERIOD COVERED

July 1, 1990 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Tumors of Rats and Mice Fed NIH-07 and NTP-90 Diets

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G.N. Rao	D.V.M., Ph.D.	CCB	NIEHS
Others:	J. Edmondson	Biologist	CCB	NIEHS

## COOPERATING UNITS (if any)

CMB

## LAB/BRANCH

Chemical Carcinogenesis Branch

## SECTION

Laboratory Animal Management

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS

1.6

## PROFESSIONAL:

0.1

## OTHER:

1.5

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The maximum mean body weights of rats attained during the course of two-year studies increased by about 20% from 1975 to 1985. Higher body weights will lead to increases in the incidences of mammary tumors, pituitary tumors, and possibly other tumors. Modification of diet may slow the growth and lower the maximum body weight attained which in turn may decrease the incidences of spontaneous tumors. Lower protein diet may decrease the incidence and severity of kidney disease. The purpose of this study is to determine the feasibility of 13% protein and 20% fiber diet (NTP-90) in lowering the maximum body weight and decreasing the severity of nephrosis in rats and mice in comparison with high protein, low fiber NIH-07 diet. Groups of 60M+60F F344 rats and B6C3F1 mice will be fed NIH-07 or NTP-90 diet for two years. Body weights, feed consumption and clinical chemistry parameters will be determined at selected intervals. Tissues will be collected for evaluation of nonneoplastic and neoplastic changes at the end of the study.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21012-09 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TERMINATED September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

## Organ and Species Differences in Chemical Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Langenbach

Microbiologist

ECMB

NIEHS

Others: K. Rudo

Biologist

ECMB

NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.4

## PROFESSIONAL:

1.4

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21013-09 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TERMINATED September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Gene Toxic/Carcinogenic Events in Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. R. Boone	Microbiologist	ECMB	NIEHS
Others:	R. W. Tennant	Supervisory Microbiologist	ECMB	NIEHS
	K. Boroto-Esoda	Biologist	ECMB	NIEHS
	C. L. Innes	Microbiologist	ECMB	NIEHS
	C. K. Heitman	IRTA Fellow	ECMB	NIEHS

## COOPERATING UNITS (if any)

Wen K. Yang, Biology Division, Oak Ridge National Laboratory

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

3.6

## PROFESSIONAL:

1.6

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21016-09 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzymes Involved in DNA Repair and Meiosis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. A. Resnick Supv. Research Geneticist ECMB NIEHS

Other: E. Perkins NRC Fellow ECMB NIEHS

## COOPERATING UNITS (if any)

Terry Chow, National Research Council, Canada

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.1

## PROFESSIONAL

0.1

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

From the studies of DNA repair and recombination using various organisms, it is apparent that nucleases play a major role in both of these processes. In *S. cerevisiae* the RAD52 gene is essential for the repair of DNA double-strand breaks, mitotic recombination and for the successful completion of meiosis. A 72 kd nuclease had been identified and subsequently purified. In rad52 mutants, levels of yNucR are greatly decreased suggesting that this nuclease is under the control of RAD52. Using a lambda gt11 expression library and an antibody which immunoprecipitates yNucR, the gene encoding yNucR has been cloned. Subsequently, a clone encoding the entire yNucR has been isolated from a YEp213 yeast genomic library. Using lacZ fusion analysis, the direction of transcription of yNucR has been determined. With the yNucR::lacZ fusion, the levels of the fusion protein has been determined during meiotic growth and in response to ionizing radiation. During meiosis, yNucR::lacZ increases approximately 2-3 fold and prior to the commitment to recombination. However, the levels of fusion protein slightly decrease in response to ionizing radiation. Western blots of wild-type yNucR in cells previously irradiated indicate that yNucR greatly decreases after irradiation (by two hours post-irradiation cross-reacting material disappears). These results suggest a proteolytic cleavage of yNucR or high turnover of the protein in response to ionizing radiation. Using the clone for yNucR, we have begun gene disruption studies of the gene. Initially, our studies indicated that gene disruption of yNucR leads to lethality. However, this lethality appears to be strain specific. Chromosome blots using the cloned yNucR indicates that this clone hybridizes to both chromosome XI and VI. Therefore, there may exist different functional copies of yNucR and the number of copies may be strain specific. Further molecular and genetic analysis is currently underway to determine the number of functional copies of yNucR encoded in the yeast genome.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21032-06 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Peroxidase Oxidation Systems in Mutation Assays

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Caspary	Biochemist	ECMB	NIEHS
Others:	D. Daston	Biologist	ECMB	NIEHS
	M. Hughes	Guest Worker	LMB	NIEHS
	T. Eling	Biologist	LMB	NIEHS

## COOPERATING UNITS (if any)

Laboratory of Molecular Biophysics, NIEHS

## LAB/BRANCH

Cellular and Genetic Toxicology Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mechanisms of metabolism other than those mediated by the mixed function oxidases may be important in activating certain chemicals to their ultimate carcinogenic form. Prostaglandin H synthetase is being used to activate compounds in mammalian cell mutation assays. Initial experiments showed hydrogen peroxide with sodium pyruvate. Using 5-phenyl-4-pentenyl hydroperoxide as a substrate, we have observed the mutagenic response to several chemicals. The possible mechanisms responsible for the formation of mutagenic metabolites induced by prostaglandin H synthetase as well as the mutation spectrum are being elucidated.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21049-08 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Synthesis and Metabolism During Meiosis

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	ECMB	NIEHS
	J. Westmoreland	Biologist	ECMB	NIEHS
	E. Perkins	NRC Fellow	ECMB	NIEHS
	A. Sugino	Visiting Scientist	LGM	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.3

## PROFESSIONAL

0.3

## OTHER

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating the unique chromosomal metabolic events associated with meiosis and the repair of chromosomes following exposure to DNA damaging agents. Many of the genes necessary for the repair of DNA double strand breaks (DSB) are required for the successful completion of the meiotic cycle. Previously, the DNA has been characterized from various stages of meiosis in both wild-type and repair deficient cells of yeast. No changes in the single-strand or double-strand size of chromosomal DNA are detected at any time during meiosis, while changes are observed in various mutants. From this, we have begun investigating the proteins/enzymes that might be involved in both repair and meiosis. We have purified two enzymes which may play a role in meiosis and/or repair; a Mg<sup>2+</sup> dependant nuclease (yNucR) and a protein that is able to carry out a strand exchange reaction (SEP). Both of these proteins appear to be under the control of the RAD52 gene, a gene required for the repair of radiation induced DSB and the completion of meiosis. This suggests that the RAD52 gene has a control function. In order to investigate the role of RAD52, we have created strains which contain complete deletions of the RAD52 gene and contain a copy of RAD52 under the control of the yeast GAL1 promoter. Thus we are able to precisely regulate the cellular levels of the RAD52 protein (and therefore DSB repair) by the carbon source in the growth media. Using this unique construct we have found that for the repair of DSB the presence RAD52 protein is required prior to treatment with ionizing radiation. We will use this "conditional" RAD52 to investigate the apparent control of yNucR and SEP.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21051-07 ECMB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Cytogenetic Analysis of Mutagen-Sensitive Mutants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James M. Mason

Geneticist

ECMB

NIEHS

## COOPERATING UNITS (if any)

University of California, Davis

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

## PROFESSIONAL:

## OTHER:

0.2

0.1

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided.)

Mutants of the mei-9 and mei-41 genes of *Drosophila melanogaster* are sensitive to a variety of mutagenic agents, defective in excision and postreplication repair respectively, and meiotic recombination, and have fragile chromosomes. The mei-41 gene is a hot spot for EMS and P-element insertion mutagenesis and shows a high frequency of interallelic meiotic recombination, suggesting that the gene is relatively large. To confirm this hypothesis and to better understand the structure and regulation of genes controlling DNA repair, these genes have been cloned using transposon tagging and are being characterized molecularly. The mei-41 transcript is 2.2 kilobase pairs in length and distributed over 14-28 kilobase pairs of genomic DNA. The mei-9 has not yet been cloned because multiple repeated sequences in the immediate region make molecular walking difficult. Another gene, mus308, has been identified based on increased sensitivity to mutagens and appears to be analogous with Fanconi's anemia, a human disorder associated with chromosome instability and an increased incidence of leukemia. This gene is also being cloned.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21053-07 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Control of Mutation in Drosophila

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: James M. Mason  
Min WangGeneticist  
Visiting FellowECMB  
ECMBNIEHS  
NIEHS

## COOPERATING UNITS (if any)

University of California, Irvine

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.8

## PROFESSIONAL:

1.4

## OTHER:

1.4

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

This project is designed to determine the relationship between DNA repair, chromosome structure and mutagenesis in *Drosophila melanogaster*. A mutation that increases the mutation frequency (a mutator) has been identified and characterized. This mutator greatly reduces the efficacy of a repair pathway for x-ray induced chromosome breaks, thereby allowing a previously undescribed repair pathway to be observed. By this newly identified repair pathway broken chromosome ends are "capped" with a new telomere. The new chromosome ends are protected from degradation by other repair mechanisms, but are not replicated effectively and DNA sequences are lost from the capped ends. The DNA at the ends of several of these chromosomes have been sequenced and found to have no DNA distal to the genomic breakpoint. This observation suggests that proper replication of the chromosomal end may require a specific telomeric DNA sequence that has been described by others, but that chromosome viability is determined by a nonDNA component of the telomere. Stable derivatives of the capped ends have been isolated and found to have gained a telomere specific DNA sequence. These stable ends are being characterized. We are also developing a rapid assay for the mutator to facilitate genetic analysis and are investigating cell specificity of mutator activity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER 201 ES 21054-07 ECMB
PERIOD COVERED October 1, 1988 to September 30, 1989		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) DNA Damage and Repair in Centromeres of Yeast		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. A. Resnick	Supv. Research Geneticist      ECMB      NIEHS
Others:	J. Westmoreland	Biologist      ECMB      NIEHS
COOPERATING UNITS (if any)  Kerry Bloom, Associate Professor, University of North Carolina, Chapel Hill		
LAB/BRANCH Experimental Carcinogenesis and Mutagenesis Branch		
SECTION		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>           Chromosome segregation requires a functional spindle apparatus, microtubules, chromosomal attachment sites, and a centromere specific DNA sequence. Disruptions of any of these organelles can lead to chromosomal malsegregation and aneuploidy. We are addressing two aspects of the function of centromeres within yeast cells: 1) the ability of cells to modify the number of centromeres; and 2) the ability of cells to deal with damage in the centromere. We developed a plasmid system which allows for the genetic detection of the number of centromere-containing plasmids within a cell. This is being done by including within a centromere plasmid the gene for copper resistance <u>CUP1</u> and a gene for <math>\beta</math>-galactosidase. Increases in plasmid number lead to increased resistance and more <math>\beta</math>-galactosidase. We have observed that haploid cells can tolerate at least 8 additional centromeres and that this does not disturb growth or the process of meiosis. This system will enable an analysis of the relationship of the spindle apparatus organization to centromere function. We have shown that toleration of extra centromeres is greatly reduced in cells of higher ploidy (i.e., diploids, triploids, and tetraploids), indicating a limitation of components for segregation. It appears that the temporary presence of large numbers of centromeres can inhibit meiosis. Because of the systems we have available for detecting aneuploidy, it will be possible to determine consequences of altered centromere number on genome stability with a high degree of detection (<math>&lt;10^{-5}</math>). Cells containing a large number of centromere plasmids are being used to examine repair in the centromere DNA.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21080-06 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nuclear Magnetic Resonance Imaging Facility

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot

Veterinary Pathologist ECMB

NIEHS

## COOPERATING UNITS (if any)

Department of Radiology, Duke Medical Center, Durham, NC

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

Cancer Genetics and Molecular Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.1

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Magnetic resonance imaging (MRI) techniques provide multidimensional images of soft tissues of the body. This non-invasive procedure is conducted on live animals and current technology permits volume resolution of 20 x 20 x 50 microns. Thus, it is possible to perform microscopy on live animals utilizing MRI techniques developed by Dr. G. Allan Johnson of Duke University. Collaborative studies conducted to date have demonstrated the utility of following progression and regression of toxic lesions in the kidney and liver of chemically treated rats. Current work is focused on development of imaging strategies to characterize specific types of lesions induced by several target organ toxicants to provide an adjunct to conventional pathologic studies and, at the same time, avoid the necessity for use of large numbers of experimental animals in toxicity studies. Collaborative studies will continue for the next year.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21091-05 ECMB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

Effects of DNA Lesions on Untargeted DNA Metabolic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. A. Resnick Supv. Research Geneticist ECMB NIEHS

Others: C. Bennett Visiting Fellow ECMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.1

## PROFESSIONAL:

1.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Inaccurate repair of DSBs can lead to such potentially lethal events as genomic rearrangements or deletion. Unrepaired DSBs can lead directly to chromosome loss and indirectly may be mediators of untargeted DNA metabolic events including enhanced recombination or replication arrest. We have utilized the site specific endonuclease HO and the MAT switching locus (YZ junction) from the yeast *S. cerevisiae* to investigate the molecular consequences of extrachromasomally induced DSBs. A 45 bp YZ junction fragment flanked by nonhomologous bacterial DNA sequences was cloned into a selectable high copy 2u vector and transformed into a nonswitching deploid yeast strain ACY522. This strain was subsequently transformed with a second selectable plasmid (pGALHOT) containing the HO endonuclease under GAL control. In vivo breakage of the YZ junction DSB cut site was monitored at various times following galactose addition using Southern blot analysis. DS breakage at the YZ junction was initially observed at 4 hours and was maximal at 10 hours following galactose addition. YZ cutting of the 2u target plasmid was incomplete with >50% supercoiled plasmid remaining at 10 hours after galactose addition. Since >80% of the cells maintain the HO plasmid pGALHOT during the galactose time course, a significant fraction of the cells with DSBs also contain unbroken plasmid. Plating of cells containing the HO and YZ target plasmids under selection to minimal galactose plates results in a severe inhibition of growth producing microcolonies of elongated filamentous cells. Glucose rescue of these microcolonies suggest that the majority of these cells are dead. Morphological analysis of unsynchronized galactose grown 2uYZ and pGALHOT containing cells show an increased number of doublet cells characteristic of a radiation induced cell cycle blockage in G2. Cytological staining of cell nuclei of doublet cells using DAPI or giemsa stains indicate a dumbbell morphology with the nucleus trapped in the bud isthmus. These results are consistent with DSBs mediating cellular arrest in trans.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21096-04 ECMB

## PERIOD COVERED

October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification and Isolation of c-fms Protooncogene from F344/N Rat Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	John E. French	Physiologist	DTRT/ECMB	NIHS
Others:	S.A. Stefansky	Pathologist	DBRA/LMT	NIHS
	C. Walker	Molecular Biologist	CIIT	

## COOPERATING UNITS (if any)

Laboratory of Molecular Toxicology, DBRA, NIEHS

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.3

## PROFESSIONAL

0.2

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☐ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Understanding the chemically induced or associated occurrence of mononuclear cell leukemia (MNCL) in NTP two-year chronic toxicology and carcinogenesis studies may be complicated by the high background rate of this tumor in aging F344 rats (20 to 30% after 24 months of age). A F344 rat leukemia transplant model has been developed to characterize the biology of this rodent leukemia and to investigate the relationship between age-related, environmental factors, and/or chemically related or modulated (promoted) leukemia. As described previously, karyotype analysis indicates that both spontaneous and serially transplanted leukemia cells have a normal complement of chromosomes (2N=42) with a variant x-subterminal chromosome, but no further details are available on non-random chromosomal changes. Sister chromatid exchange rates in bone marrow cells of aging Fischer 344 rats are ~1.5 times greater than Wistar rats and the background tumor incidence is similarly increased. Studies are in progress to define non-random chromosomal changes (additions, deletions, translocations, etc.) and using high-resolution G-banding techniques and the relationship to the expression of the c-fms protooncogene product (for hematopoietic growth factor receptor, CSF-1). Final results on the identification of c-fms indicate that these rat leukemia cells from both spontaneous (7/7, untreated, >22 months of age) and transplanted (8/8) leukemias express the fms/CSF-1 receptor as 3.8 kb RNA transcript identical to that expressed by normal rat macrophages. This information is important to the diagnosis and understanding of chemical effects in this rodent model as a surrogate for human exposure to toxic and/or carcinogenic chemicals.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21106-03 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In situ Protocols for Mammalian Cell Mutagenesis Assays

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Caspary	Biochemist	ECMB	NIEHS
Others:	D. Daston	Biologist	ECMB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

1.5

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An in situ protocol for mammalian cell mutagenesis assays, in which cells are fixed during the expression and selection phases, was developed. It allows for the calculation of the mutation frequency, the proportion of new mutations in a population of cells, and rather than the mutant fraction, the proportion of mutants in a population of cells. The mutant fraction can give misleading assessments of the mutagenic activity of chemicals when a large number of mutants grow more slowly than the rest of the population. This protocol permits the calculation of mutation rates and we anticipate that it will give a more accurate assessment of the mutagenic activity of chemicals than standard protocols.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21121-02 ECMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transfection of cDNAs for Drug Metabolizing Enzymes into Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Langenbach	Microbiologist	ECMB	NIEHS
Others:	H. Tiano	Biologist	ECMB	NIEHS
	P. Smith	Visiting Scientist	ECMB	NIEHS
	M. Hosokawa	Visiting Associate	ECMB	NIEHS

COOPERATING UNITS (if any)

Dr. S. Nesnow, U.S. EPA, Research Triangle Park, NC

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS.

2.4

PROFESSIONAL

2.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The metabolism capability of mammalian cells used in mutation and transformation assays is being increased by infecting the cells with retroviral vectors containing cDNAs coding for drug metabolizing enzymes. The cDNAs for P450s IIA3, IIB1, and IVB1, and a flavin monooxygenase have been inserted into the vectors and infected into C3H 10T½ cells. By Western analysis, all p450s and the flavin monooxygenase are expressed in the 10T½ cells. Furthermore, cells containing IIB1 have increased sensitivity to the cytotoxic effects of dimethylnitrosamine and acetylaminofluorene, and cells containing IVB1 show increased cytotoxic effects to aminofluorene. Cytochrome P450 IIA3 is detected by coumarin hydroxylase activity and should activate the carcinogens, dimethyl- and diethylnitrosamine. The studies are continuing to improve enzyme expression and to make the cells responsive to the genetic toxicity of a wider variety of environmental chemicals and to mimic carcinogen activation as it occurs in vivo.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21122-02 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genomic Stability and Recombinational Interactions

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	ECMB	NIEHS
	G. Porter	I.R.T.A.	ECMB	NIEHS
	Scott Priebe	N.R.C. Fellow	ECMB	NIEHS
	M. Radman	Visiting Scientist	LMG	NIEHS

## COOPERATING UNITS (if any)

T. Nillsson-Tillgren, University of Copenhagen, Denmark

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.4

## PROFESSIONAL:

0.4

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recombination is required for the repair of many types of lesions and it can be a source of genetic diversity. We are investigating the requirements for homology in recombination and the consequences of recombination between DNA divergent sequences. From this information we can determine the mechanisms of chromosome rearrangements, generation of novel genes and possible mechanisms of initiation in carcinogenesis. In addition we are developing a system for the genetic detection of double-strand damage after exposure to very low, nonlethal doses of an agent. We developed a method for examining the role of homology between a specific pair of homologues in "protecting" chromosomes against DNA damage. Nonlethal radiation doses to *S. cerevisiae* diploid cells containing a single pair of DNA divergent (80% homologous) but functionally homologous chromosomes greatly increased aneuploidy induction (chromosomes III or V from *S. cerevisiae* and *S. carlsbergensis*). Using these approaches, we have investigated the fate of damaged human DNA contained in yeast vectors in yeast (YACs). Provided there is an homologous YAC, repair is efficient but there is little or none in the absence of a homologue. We are investigating the possibility of recombination between repetitive sequences in the human DNA. We have concluded that recombinational repair can occur between sequences of limited homology. This was also demonstrated by radiation induced intragenic recombination between homoeologous chromosomes. We have also shown that gene targeting in yeast can occur with limited homology. Since mismatch repair would be expected to play an important role, we have begun to investigate model heteroduplex transforming molecules in various yeast and *E. coli* mutants.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21129-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Frozen Tissue Archives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot

Veterinary Pathologist ECMB

NIEHS

## COOPERATING UNITS (if any)

EPL. Inc. (Contractor for NTP Archives)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

Cancer Genetics and Molecular Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.5

## PROFESSIONAL:

0.5

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Representative samples of tumor tissue and appropriate normal tissues are being collected at the terminal sacrifices of National Toxicology Program rat and mouse carcinogenesis bioassays and frozen for subsequent molecular biology studies. Studies to identify activation of oncogenes and loss or alteration of tumor suppressor genes in these samples are underway. Samples are being collected for use by various NIEHS investigators. Special efforts will be made to obtain chemically induced tumors when it is apparent that a carcinogenic effect is being manifested. Work on these samples as well as similar samples collected from in-house and other contractor-generated tissues has been underway for the past 5 years and has yielded information helpful for interpretation of bioassay results and useful in risk assessment. It is anticipated that collection efforts will continue over the next 4 years.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21130-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphogenesis of Pulmonary Neoplasia in Mice

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Darlene Dixon

Pathologist

ECMB

NIEHS

Others: R. R. Maronpot

Veterinary Pathologist ECMB

NIEHS

## COOPERATING UNITS (if any)

Division of Biometry and Risk Assessment, NIEHS

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

Cancer Genetics and Molecular Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

2.5

## PROFESSIONAL

1.25

## OTHER:

1.25

## CHECK APPROPRIATE BOXES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies designed to assess the histomorphological, ultrastructural, histochemical and biochemical characteristics of altered cell types in pulmonary neoplasms of mice are currently underway. Over the past year, we have evaluated the gross, histomorphological and ultrastructural characteristics of pulmonary tumors in aged Strain A mice in an attempt to further clarify differences between neoplastic and nonneoplastic lung tissue using conventional light and electron microscopy. Automated lectin staining, two dimensional gel electrophoresis and gold-labeling electron microscopic techniques to evaluate glycoconjugate composition of nonneoplastic versus neoplastic cells, and malignant versus benign tumors, are in progress. Additional studies to characterize the progression of pulmonary neoplasia in other strains of mice including B6C3F1, C3A, C57BL, C3H, B6D2F1 and AC3 utilizing standard morphological and morphometric techniques are underway.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21131-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Chemically Induced and Spontaneous Neoplasms for Oncogene Activation

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot

Veterinary Pathologist ECMB

NIEHS

Others: M. W. Anderson

Research Chemist

LMT

NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

Cancer Genetics and Molecular Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Oncogene studies on spontaneous and chemically induced rodent neoplasms, started 5 years ago in collaboration with molecular biologists in the Division of Biometry and Risk Assessment, are continuing. Emphasis has been on the patterns of oncogene activation in chemically induced versus spontaneous lung and liver neoplasms using samples from NTP bioassays as well as from rodent studies designed to investigate strain susceptibility to genotoxic and nongenotoxic chemical carcinogens. Important information useful for risk assessment and understanding mechanisms of carcinogenesis has been generated from past studies and underscores the need to continue these studies over the next two to three years.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21132-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Image Analysis, Quantitative Morphometrics and Cell Turnover

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: R. R. Maronpot

Veterinary Pathologist ECMB

NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

Cancer Genetics and Molecular Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.5

## PROFESSIONAL

0.1

## OTHER

0.4

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Image analysis of pathologic specimens with emphasis on quantitation of cell proliferation has been underway over the past two years. The focus has been on utilizing state-of-the-art computer software to quantitate cell turnover in liver and lung tissues following exposure to genotoxic and nongenotoxic carcinogens. Development of immunohistochemical procedures for demonstrating S-phase nuclei following administration of bromodeoxyuridine offers great promise as a useful technique. In addition, new methods for demonstrating proliferating cell nuclear antigen have recently been established in our laboratory. The new techniques are being applied to tissues of mice exposed to methylene chloride by inhalation for varying periods of time, including a two-year carcinogenesis study. Standard morphometric techniques are being used to chart development and growth of preneoplastic lesions and tumor progression. This work will continue for the next two years.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21133-01 ECMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparative Genetic Toxicology of H.C. Blue 1 and H.C. Blue 2

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robert Langenbach	Microbiologist	ECMB	NIEHS
Others:	Frank Kari	Biologist	STB	NIEHS
	Amal Abu-Shakra	Visiting Fellow	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

SECTION

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

H.C. Blue 1 is carcinogenic in the B6 mouse liver but not in the F344 rat, whereas H.C. Blue 2 is not carcinogenic in either species. The in vivo metabolism of the chemicals in the urine of both species, and the in vitro metabolism by hepatocytes from both species, have been analyzed. In vivo metabolism and hepatocyte metabolism gave similar HPLC profiles for each chemical. Rats and mice differed quantitatively in the H.C. Blue 1 metabolite profiles produced, which may contribute to the H.C. Blue 1's species specificity. The identity of specific metabolites are currently being identified. The adduction to DNA of H.C. Blue 1 to mouse and rat liver tissue had hepatocytes has been investigated. But studies have indicated an impurity(ies) may be responsible for adduct formation and therefore H.C. Blue 1 has been subjected to HPLC purification. From these studies, an impurity in H.C. Blue 1 responsible for mutagenicity and DNA adduction in Salmonella has been isolated. Seven DNA adducts have been identified in Salmonella. Purified H.C. Blue 1 is not mutagenic and does not form DNA adducts. Studies are continuing in an attempt to elucidate the basis of H.C. Blue 1's carcinogenic activity in the mouse and lack of carcinogenic activity in the rat.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21139-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Mutagenesis and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Caspary	Biochemist	ECMB	NIEHS
Others:	D. Daston	Biologist	ECMB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

.5

## PROFESSIONAL

.5

## OTHER

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Slowly growing mutants have hampered the assessment of the mutagenic effect of chemicals. A new, in situ protocol allows for the inclusion of slowly growing mutants in the measurement of the mutagenic response. Inclusion of these slowly growing mutants increases the measurement of the mutation rate by 50-fold over old techniques. These new techniques allow for a more accurate measurement of the molecular lesions induced by chemicals and these lesions are being related to the DNA adduct induced by these chemicals.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21140-01 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of an In Vitro System for Nonmutagenic Carcinogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. W. Tennant Supervisory Microbiologist ECMB NIEHS

Others: C. L. Innes Microbiologist ECMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

.2

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The development of an in vitro assay system for nonmutagenic carcinogens is the primary focus of this laboratory. The system involves the use of undifferentiated embryonal carcinoma cells which are known to undergo changes in transcription and gene expression when exposed to certain chemicals. Two of these changes, cell differentiation and provirus transcription/expression, are being studied as assay endpoints. The goal is to determine if the ability of a chemical to cause neoplasia is related to its ability to cause either of the above changes.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 60122-11 ECMB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of DNA Repair in Yeast and Their Role in Meiosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Resnick	Supv. Research Geneticist	ECMB	NIEHS
	G. Porter	I.R.T.A.	ECMB	NIEHS
	Scott Priebe	N.R.C. Fellow	ECMB	NIEHS
	M. Radman	Visiting Scientist	LMG	NIEHS

## COOPERATING UNITS (if any)

Dr. J. Mittiss, Harvard University, Cambridge, MA

Dr. J. Game, University of CA, Berkeley, CA

## LAB/BRANCH

Experimental Carcinogenesis and Mutagenesis Branch

## SECTION

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

1.1

## PROFESSIONAL

0.1

## OTHER

1.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DNA repair systems identified in mitotic cells of the yeast Saccharomyces cerevisiae are being examined for their protection of cells undergoing meiosis and the role of the corresponding genes in normal meiosis. The RAD50, RAD52 and RAD57 genes are essential in the repair of DNA double-strand breaks in mitotic cells. We have shown that they are also required for meiosis. Mutations abolish normal meiotic recombination; RAD50 acts early in meiosis. Rare single-strand interruptions (SSIs) are observed in rad52 and rad57 strains which appear to be related to recombination and these have been characterized. Based on genetic and biochemical changes, the order of gene function appears to be RAD50, RAD52, and RAD57. Given the important role RAD52 plays in repair and recombination, we have initiated studies to characterize its function in normal DNA metabolism and following treatment with DNA damaging agents. This is being done by "domain mapping" the functional regions of the RAD52 gene and by examining its interactions with other genes. A complete deletion has been created and modified complementing and mutant versions of the RAD52 are maintained on a plasmid. Using this system in combination with a mutation in the DNA polymerase III gene, the RAD52 has been shown to be required during replication. The RAD52 gene product is also essential in repair and must be present prior to DNA damage exposure.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21003-10 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disposition of Halogenated Dibenzofurans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Linda S. Birnbaum	Research Biologist	DTRT	NIEHS
Others:	Yolanda Banks	Biologist	DTRT	NIEHS
	Lorrene Kedderis	Guest Researcher	DTRT	NIEHS
	Janet Diliberto	Biologist	DTRT	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

2.1

## PROFESSIONAL:

0.1

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are toxic environmental and industrial contaminants. Dermal absorption constitutes a major route of exposure to these chemicals with maximal absorption of a low dose ranging from 40% (TCDD) to 66% (TCDF). Absorption through the skin occurs very slowly yet appears to be more rapid during the first 24 to 48 hours after contact. Our results with TCDD and TCDF suggest that physicochemical properties may govern the rate of absorption of these chemicals. Absorption of a low dose of TCDD through the skin of very young animals is increased compared to absorption through the skin of adult animals. Our results suggest that the potential for systemic exposure might be less in adults compared to children.

Polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PCDFs) are potential environmental contaminants due to their formation from thermolysis or pyrolysis of certain brominated flame retardants. The disposition of TBDD was studied in male F-344 rats following oral or intravenous administration. TBDD exhibited non-linear absorption kinetics with maximal oral absorption (~80%) occurring at doses < 0.01 umol/kg. Distribution to the liver and adipose, the major tissue depots, was dose-dependent, with preferential accumulation in the liver versus the adipose at higher doses. Feces was the major route of elimination and excretion was dose-dependent. The apparent terminal whole body half-life of TBDD was estimated to be 18 days. Within 6 hours, ~6% of the dose was excreted into the bile in metabolized form; pretreatment did not appear to enhance biliary excretion. The overall disposition of TBDD appears similar to that observed for TCDD. The dose-dependent tissue distribution and excretion kinetics suggest important considerations for high to low dose extrapolations.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21004-10 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Senescent Changes in Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Linda S. Birnbaum Research Microbiologist DTRT NIEHS

Others: Yolanda Banks	Biologist	DTRT	NIEHS
Timothy McMahon	IRTA Fellow	DTRT	NIEHS
Janet Diliberto	Biologist	DTRT	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.4

## PROFESSIONAL:

1.1

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The elderly represent a special population who are often at increased risk for susceptibility to the toxic effects of environmental agents. Alterations in disposition and/or biotransformation of xenobiotics in aged subjects may be a contributing factor to this enhanced susceptibility. In this laboratory, work is focused on alterations occurring with age in absorption, distribution, metabolism, and excretion of environmental toxicants, using two rodent models of aging. Benzene is a widespread environmental contaminant and human carcinogen whose disposition and toxicity has not been previously assessed in elderly subjects. Disposition and metabolism of benzene were examined in aged mice at 10 and 200 mg/kg. In addition, a physiologically based pharmacokinetic model was used to gain further insight into the basis of age-related differences in benzene disposition, and implications for toxicity of benzene in older subjects. Disposition of benzene was altered with age, in that older mice appeared to retain more of a given dose of benzene. Physiologically based pharmacokinetic modeling of benzene disposition suggested that this alteration could be due to changes in total benzene metabolism or in absorption of benzene. Verification of these model predictions is currently under investigation.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21033-06 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990 TERMINATED September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disposition of Xenobiotics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: L. S. Birnbaum Research Microbiologist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (a1) Minors

☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01-ES 21038-08 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical Metabolism and Disposition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dr. H.B. Matthews Research Chemist ETB NIEHS

Others: L.T. Burka Research Chemist ETB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Chemical Disposition

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

0.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unlined type. Do not exceed the space provided.)

Studies of chemical metabolism and disposition are designed to provide both applied knowledge of the fate of chemicals in intact animals in support of toxicity tests conducted by the National Toxicology Program and basic knowledge of mechanisms of chemical toxicity. These studies are designed to determine the absorption, tissue distribution, metabolism and clearance of chemicals and the effect of such factors as dose and route of exposure on each of these parameters. Tris(2-chloroethyl)phosphate (TRCP), a flame retardant plasticizer used widely in industrial and consumer products, has been demonstrated to cause an unusual lesion in the hippocampus of rats administered this compound orally. The hippocampal lesion was more pronounced in female than male rats and was not observed in mice. Studies to characterize the fate of TRCP in male and female rats and mice have established that this compound is readily absorbed and distributed systemically, that metabolism is rapid and that the major metabolite is somewhat unique. The major metabolite has been identified as bis(2-chloroethyl) carboxymethyl phosphate and the second major metabolite has been identified as bis(2-chloroethyl) hydrogen phosphate. Neither of these metabolites is thought to account for the neurotoxicity of TRCP, rather it appears that the greater sensitivity of female rats may be due to the fact they clear this compound more slowly in the first few minutes following dosing. In a study of the fate of oxymetholone (OXM), a synthetic androgen, it was determined that this compound is absorbed from the gastrointestinal tract at a moderate rate to result in peak blood levels 2 to 4 hr. post oral dosing. It was also determined that OXM is metabolized and eliminated in bile, 35% in 7 hr. and that 80% of the dose eliminated in feces in 72 hr.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21070-07 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TERMINATED September 30, 1989

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

TCDD Teratogenicity: Modulation in Mixtures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: L. S. Birnbaum Research Microbiologist ETB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.6

## PROFESSIONAL:

0.2

## OTHER:

1.4

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21075-07 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Xenobiotic Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	L. T. Burka	Chemist	DTRT	NIEHS
Others:	Devendra Parmar	Visiting Fellow	DTRT	NIEHS
	Robert Chapin	Toxicologist	DTRT	NIEHS
	Diane Overstreet	Chemist	DTRT	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.6

## PROFESSIONAL:

1.2

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors        |  |   |
| <input type="checkbox"/> (a2) Interviews    |  |   |

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Understanding how a xenobiotic is metabolized, distributed, and eliminated is often critical to an appreciation of the toxic effect(s) of the compound. Further, extrapolation of results from animal testing to possible human health effects requires knowledge of metabolic pathways. The fidelity of the extrapolation is enhanced if the fate of a xenobiotic is known for both (all) species used in the extrapolation. Investigation of the mechanistic aspects of metabolic processes allows greater understanding of how metabolism of a xenobiotic might lead either to detoxification or to a reactive metabolite with greater toxicity. As more is learned about mechanisms of metabolism, more accurate predictions of the possible metabolic pathways for new compounds should be possible. This group has carried out studies on 1,2,3-trichloropropane (TCP), saligenin cyclic o-tolyl phosphate (SCOTP), cyclohexanone oxime (CHOX) and furan in the past year. As part of our continuing study on the metabolism of TCP, an industrial solvent, we have identified 2-(S-glutathionyl) malonic acid as a biliary metabolite. This metabolite could arise by a series of oxidations and episulfonium ion intermediates and points out the potential reactivity of this compound. The male reproductive toxicity of tri o-cresyl phosphate may be attributed to the metabolic formation of SCOTP. In a recently completed study to determine the in vivo half-life of this reactive compound, the blood half-life of SCOTP was found to be long enough to be formed in the liver and transported to the testes--the target tissue. CHOX was readily absorbed, distributed and eliminated after a single oral dose of 1, 10 or 30 mg/kg. The majority of the radioactivity was eliminated in 24 hr. Pharmacokinetic studies have shown that CHOX has a biological half life of less than 1 hr. Only 4-5% of a dermally administered dose was absorbed. Oral administration of furan decreased hepatic P-450 content and the activity of P-450-dependent enzymes. Metabolism dependent covalent binding of furan to microsomal protein indicates that P-450 IIE1 preferentially catalyzes the metabolic activation of furan.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21076-07 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990 TERMINATED September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Biochemistry Studies on Chemicals Selected for Evaluation by NTP

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael P. Dieter	Physiologist	ETB	NIEHS
Others:	C.W. Jameson	Chemist	ETB	NIEHS
	M.D. Shelby	Head, Mammalian Mutagenesis	ECMB	NIEHS
	G.A. Boorman	Pathologist	CCB	NIEHS

## COOPERATING UNITS (if any)

Experimental Carcinogenicity and Mutagenesis Branch  
Chemical Carcinogenesis Branch

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

General Toxicology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC

## TOTAL MAN-YEARS:

0.5

## PROFESSIONAL:

0.4

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

I.P. injection studies with sodium chromate, zinc potassium chromate, and chromium carbonyl were conducted in mice to compare the genotoxic effects of the hexavalent salts with their potential myelotoxic effects. At the doses employed, there were no significant elevations of micronucleus frequencies caused by any of the three chromate salts. Studies of the absorption and target organ toxicity of antimony potassium tartrate were completed in rats and mice given i.p. injections for 7 or 14 weeks. Dose-related levels of antimony accumulated in the liver, blood, kidney, spleen, and heart, but there were no biochemical changes indicative of toxicity except in the liver. Hepatocyte degeneration and multifocal liver degeneration occurred in association with dose-related elevations in activities of the liver-specific serum enzymes, sorbitol dehydrogenase and alanine aminotransferase. Alteration of the site of injection and the days of treatment demonstrated that the i.p. route could be practically used as a route of administration in 2-year studies. Similar studies were conducted in rats and mice administered ferrocene by inhalation for 14 or 90 days. Lung burdens of iron were increased in proportion to dose and time. The only toxic response was subacute, necrotizing inflammation of the olfactory epithelium in the nasal turbinates of both species after 14 days administration, and degeneration, inflammation, hyperplasia, and metaplasia (rats only) after 90 days administration. Studies terminated.



**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-ES-21084-05 ETB

**PERIOD COVERED**

October 1, 1989 to September 30, 1990

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the borders.)

Association of Chemically-Induced Cell Proliferation and Carcinogenesis

**PRINCIPAL INVESTIGATOR** (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Burhan I. Ghanayem	Toxicologist	ETB	NIEHS
Others:	M. L. Cunningham	Senior Staff Fellow	ETB	NIEHS
	H. B. Matthews	Research Chemist	ETB	NIEHS
	R. R. Maronpot	Vet Pathologist	ECMB	NIEHS

**COOPERATING UNITS** (if any)

Experimental Carcinogenesis and Mutagenesis Branch

**LAB/BRANCH**

Experimental Toxicology Branch

**SECTION**

Chemical Disposition

**INSTITUTE AND LOCATION**

NIEHS, NIH, Research Triangle Park, North Carolina 27709

**TOTAL MAN-YEARS:**

0.2

**PROFESSIONAL:**

0.1

**OTHER:**

0.1

**CHECK APPROPRIATE BOX(ES)**

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

**SUMMARY OF WORK** (Use standard unredacted type. Do not exceed the space provided.)

Chemically-induced neoplasia is a major public health concern and is the driving force behind much of the research conducted by the NTP. Present work has focused on mechanisms of chemically-induced forestomach carcinogenesis. One area of interest has been the relationship between chemical-induced early cell proliferation and carcinogenicity. Ethyl acrylate (EA) was selected as a model chemical for these studies because chronic gavage administration of EA resulted in a dose- and concentration-dependent increase in the incidence of forestomach (FS) neoplastic lesions in both sexes of F344 rats and B6C3F1 mice. No neoplastic lesions were found at any other site. The current work investigated the correlation between the induction of cell proliferation in the male rat FS (target) and liver (nontarget) and the carcinogenicity of EA. Cell proliferation was measured by assessing bromodeoxyuridine (BrDU) incorporation into DNA administered by osmotic minipump concurrent with gavage treatment with EA (50, 100 or 200 mg/kg/day) for 2, 4, or 8 days. BrDU incorporation was detected immunohistochemically. Results of these studies indicated that EA induced epithelial cell proliferation in the FS was dose- and time-dependent. Minimal or no cell proliferation was detected in the livers of EA-treated rats. These results suggest a positive correlation between cell proliferation in the target tissue and carcinogenicity.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21093-04 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Dioxin Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Linda S. Birnbaum Research Microbiologist DTRT NIEHS

Others:	Charles Hebert	Biologist	DTRT	NIEHS
	Barbara Abbott	IRTA Fellow	DTRT	NIEHS
	Laurie Couture	Biologist	DTRT	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.8

## PROFESSIONAL:

0.8

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

TCDD has a broad range of toxic effects which are both species and tissue specific and may involve interference with normal regulation of cell growth and differentiation. TCDD can modulate the levels of receptors for glucocorticoids, estrogens, and epidermal growth factor. During development, TCDD causes increases in the EGF receptor in both the medial epithelium of the palate and the ureteric epithelium, and causes the medial epithelium to differentiate into an oral epithelium rather than transform into mesenchyme and the ureteric epithelium to undergo hyperplasia. These effects, which result in cleft palate and hydronephrosis in vivo, can be achieved in organ culture of the developing palatal shelves and the urinary tract, allowing for species comparison. The lack of cleft palate induction in the developing rat fetus following TCDD exposure is due to lower sensitivity of the target fetus as compared to the mouse since in culture, rat palatal shelves can be affected by high concentrations of TCDD. In vivo, these doses are maternally toxic. The relative sensitivity of human embryonic tissue can also be explored by this method. TCDD induces proliferation of human squamous carcinoma cells apparently as a result of a failure of the cells to undergo high density growth arrest rather than a direct mitogenic stimulus. Some TCDD effects seen in these cell lines, such as induction of EROD activity, can be blocked by the addition of TGF $\beta$ , a potent growth regulator. TCDD, however, does not effect binding of TGF $\beta$  to cells, secretion of TGF $\beta$  by these cells or responsiveness of these cells to exogenously added TGF $\beta$ . The mechanism by which TCDD induces hydronephrosis has been investigated. Hyperplasia of the epithelial lining of the ureter results in occlusion of the lumen and restricts flow of urine, resulting in hydronephrosis and hydronephrosis. This effect correlates with increased ureteric epithelial expression of EGF receptors and DNA synthesis as indicated by increased tritiated thymidine incorporation.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutagenesis and Other Cellular Responses to Chemicals that Generate Free Radicals

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Errol Zeiger	Supervisory Microbiologist	ETB	NIEHS
Others:	Dennis Pagano	Microbiologist	ETB	NIEHS
	A.-A. Stark	Visiting Fellow	ETB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Mutagenesis Group

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

## TOTAL MAN-YEARS:

0.7

## PROFESSIONAL:

0.4

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The formation of hydrogen peroxide and free radicals from (glutathione) GSH is dependent on the activity of g-glutamyltranspeptidase (GGT), an enzyme that is frequently present in high amounts in preneoplastic cells. GSH, in the presence of GGT, can induce lipid peroxidation in vitro, using linolenic acid or linoleic acid as substrates, and in situ in cultured human hepatoma cells. The reaction requires iron, a chelator, GSH, and GGT, is accelerated by GGT activation factors, and is inhibited by specific GGT inhibitors and free-radical scavengers. Peroxidation occurs in the presence of physiological concentrations of iron, and endogenous chelators (citrate, ADP, transferrin). Unlike the mutagenic effect of GSH which is mediated through the formation of H<sub>2</sub>O<sub>2</sub>, lipid peroxidation appears to be effected through a free radical reaction, without the intermediate formation of H<sub>2</sub>O<sub>2</sub>. The effects of antioxidants and free radical scavengers on mutagenicity and lipid peroxidation induced by other sulfhydryls, such as D- and L-cysteine and D- and L-penicillamine will be studied. Studies are in progress to test whether lipid peroxidation can be induced in GGT-rich preneoplastic foci in rodents when challenged with GSH.

Studies are under way to validate the mutagenicity results of GSH in AS52 cells, optimize the test protocol, and to examine the effects of antioxidants and free radical scavengers on GSH mutagenicity. These cells will be used to test the mutagenicity of other substances known, or hypothesized, to form oxygen radicals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
201-ES-21097-04 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Chemical Myelotoxicity using an in vivo Leukemia Transplant Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Principal Investigator M.P. Dieter Physiologist ETB

Collaborative Investigators: C.W. Jameson Chemist ETB  
M.R. Elwell Pathologist ETB

COOPERATING UNITS (if any)

None

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.4

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Spontaneous mononuclear cell leukemia is a confounding factor in evaluating chemical leukemogenicity in the NTP 2-year carcinogenicity studies. A short-term assay for F344 rat leukemia was developed to better discriminate between age-induced and chemically-enhanced leukemia. The accuracy and sensitivity of the transplant model for predicting the long-term leukemogenic potency of chemicals was confirmed in short-term assays with 7 chemicals that had increased or decreased the prevalence of leukemia in previous 2-year carcinogenicity studies. Additional studies with the short-term assay revealed structure-activity relationships for chemicals that were either negative or positive for leukemic trends. Nine different glycol ethers were evaluated in the short-term assay for anti-leukemic activity. Of these, only ethylene glycol monomethyl ether and ethylene glycol monoethyl ether exhibited chemotherapeutic potential. Ethylene glycol monomethyl ether was a more potent antileukemic agent than the monoethyl ether, and at non-toxic doses in drinking water completely eliminated the early manifestations of leukemia, prevented early mortality, and doubled the tumor latency period. These data were confirmed by in vitro tests with suspended leukemic cell cultures. Presently, the chemotherapeutic potential for the acid and aldehyde metabolites of ethylene glycol monomethyl ether, as well as chemicals in the propylene glycol monomethyl glycol alkyl ether series, are being evaluated. By contrast, two chemicals containing dimethyl esters of phosphoric acid (dichlorvos and trichlorfon) enhanced the expression of leukemia in the short-term assay and in 2-year carcinogenicity tests. Three other chemicals with the same structural relationship: dimethyl hydrogen phosphite, dimethyl methylphosphonate, and dimethylmorpholinophosphoramidate also increased the incidence of leukemia in recently completed 2-year studies. These observations suggest that the dimethyl phosphoric acid ester moiety should be considered a structural alert for leukemogenicity. Presently, the leukemogenic potential of acetaminophen is being evaluated in the short-term leukemia transplant model to confirm and extend the observations obtained from 2-year studies.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
201 ES 21103-04 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluations of Genetic Toxicity Test Results

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Errol Zeiger, Ph.D. Supervisory Microbiologist ETB NIEHS

Others: Beth Anderson Biologist ETB NIEHS  
Walter Piegorsch Mathematical Statistician SBB NIEHS  
Barry Margolin Dept. Biostatistics, UNC

COOPERATING UNITS (if any)

Statistics and Biomathematics Branch  
Department of Biostatistics, UNC, Chapel Hill, NC

LAB/BRANCH

Experimental Toxicology Branch

SECTION

Mutagenesis Group

INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A database of results has been created using chemicals tested by the NTP for mutagenesis in short-term assays, and for carcinogenesis. This database allows the evaluation of each short-term assay with respect to its ability to predict carcinogenesis or other short-term assay results. It also permits studies of the individual assays with respect to inter and intra-laboratory reproducibility, and the effects of protocol changes on test responses.

A study is underway to examine the predictivity of the four short-term tests as a function of the potency of the short-term test and carcinogenicity test responses. The chemicals to be evaluated include the 73- and 41-chemical datasets studied earlier in a project to correlate short-term test results with carcinogenicity results, based on qualitative results. As a result of that project, the same chemicals, in the same test systems, will be examined to determine how well the potencies of the responses correlate.

A part of this study involves the development of measures of potency for the different tests. Such measures have been proposed by others for the Salmonella and carcinogenesis assays, but there are no commonly-accepted measures of potency for the other short-term tests, such as in vitro chromosome aberrations, in vitro SCE, or the mouse lymphoma assay. These measures of potency are being developed and evaluated, and will be used for the correlational studies. Different potency measures for the Salmonella test have been compared; the procedures developed by Margolin et al. and Bernstein et al. which model an initial slope give comparable values. Procedures that provide measures other than slopes will be evaluated, and one of these procedures will be used in conjunction with the slope procedures.





**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT**

**PROJECT NUMBER**  
Z01 ES 21107-03 ETB

**PERIOD COVERED**

October 1, 1989 to September 30, 1990

**TITLE OF PROJECT** (80 characters or less. Title must fit on one line between the borders.)

Mutagenicity Studies of Hydrogen Peroxide and Hydrogen Peroxide Generating Systems

**PRINCIPAL INVESTIGATOR** (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Amal Abu-Shakra	Visiting Fellow	ETB	NIEHS
	Errol Zeiger	Supervisory Microbiologist	ETB	NIEHS

Others:	Dennis Pagano	Microbiologist	ETB	NIEHS
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**COOPERATING UNITS** (if any)

**LAB/BRANCH**

Experimental Toxicology Branch

**SECTION**

Mutagenesis Group

**INSTITUTE AND LOCATION**

NIEHS, NIH, Research Triangle Park, NC 27709

**TOTAL MAN-YEARS:**

0.8

**PROFESSIONAL:**

0.6

**OTHER:**

0.2

**CHECK APPROPRIATE BOX(ES)**

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

**SUMMARY OF WORK** (Use standard unreduced type. Do not exceed the space provided.)

In the newly-constructed Salmonella strains SB1111 (hisC3018) and SB1106 (hisC3108, hisO1242), the mutagenic effects of H2O2 were observed at doses similar to those used with other strains. This C3108 mutation is reverted by H2O2 in the absence of the uvrB deletion, pKM101 plasmid, or the rfa mutation, which appear necessary for mutagenesis in strains TA97, TA102, and TA104. Incorporation of the pKM101 plasmid enhanced the reversion of SB1111 and SB1106 (SB1111p and SB1106p). SB1106p is comparable to TA104 in its spontaneous reversion rate, but is more sensitive to H2O2 mutagenesis. The spontaneous rate of SB1111p, on the other hand, was too high for routine use. Strain SB1106p is being used to test other chemicals, such as thiols, that may act through the formation or release of H2O2, to determine its utility for routine chemical testing.

The use of electrochemical detection coupled with HPLC procedures allow the effective monitoring of DNA for the induction of oxygen-damaged products, such as 8-hydroxyguanosine (8-OH-dG). This technique has been used to monitor the induction of 8-OH-dG in purified DNA by H2O2 or glutathione. In the presence of a Fenton reaction mix, glutathione induced a doubling of the background rate of 8-OH-dG in purified deoxyguanosine. 8-OH-dG was also detected in DNA samples subjected to various enzymatic digestion techniques. The levels of 8-OH-dG in DNA from calf thymus, rat liver, or Salmonella is high enough to mask low levels of induction by chemicals. New methods for DNA extraction and digestion are being adopted in an attempt to lower the background levels of 8-OH-dG.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21109-03 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of 2-Butoxyethanol Induced Hematotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Burhan I. Ghanayem Toxicologist ETB DTRT

Others: Idalia Sanchez Biol. Lab. Tech. ETB DTRT  
Sandra Ward Biol. Lab. Tech. ETB DTRT

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Early work demonstrated that 2-butoxyethanol (BE) causes acute hemolytic anemia in rats. Treatment of rats with BE daily (125 mg/kg/day) for three consecutive days resulted in a time dependent increase in the hemolysis of erythrocytes. However, when daily treatment continued beyond 3 days, the number of erythrocytes started to increase and approached the pretreatment level within 10 days despite continued daily dosing, suggesting tolerance development to the hemolytic effect of BE. To investigate the mechanism of tolerance development, rats were treated with 125 mg BE/kg/day for 3 days and allowed to recover (with no treatment) for 7 days. Control rats were treated with water similarly. At the end of this recovery period, rats were treated with 125 or 250 mg BE/kg and the hematology profiles were assessed. A significant decline in the sensitivity of pretreated rats compared to vehicle controls were observed. Furthermore, incubation of blood obtained from the recovered rats with the hematotoxic metabolite of BE, 2-butoxyacetic acid (BAA), in vitro indicated that there was a decline in the sensitivity of these erythrocytes as well. In another series of studies, the effect of BE on erythrocytes morphology in vivo and the effect of BAA on human and rat erythrocytes in vitro was investigated. Both BE and BAA caused stomatocytosis and vesiculation in rat erythrocytes. In contrast, no such effects were seen on human erythrocytes.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21117-02 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Calcium in Chemical-Induced Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Burhan I. Ghanayem Toxicologist ETB NIEHS

Others: H. B. Matthews Research Chemist ETB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.2

## PROFESSIONAL:

0.1

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Administration of 2-butoxyethanol (BE) or 2-methoxyethanol to rats by gavage induced dose-dependent acute hemolytic anemia and testicular toxicity, respectively. Early reports from this laboratory indicated that calcium channel blockers protect against the testicular toxicity of 2-methoxyethanol. In the present work, the effect of calcium channel blockers against BE-induced hemolytic anemia was investigated. Treatment of rats with calcium channel blockers, verapamil (40 mg/kg; ip) or diltiazem (90 mg/kg; ip) prior to BE resulted in a significant decrease in erythrocytic swelling, ATP depletion, and ameliorated the subsequent BE-induced hemolytic anemia. In vitro, addition of verapamil or diltiazem, at concentrations ranging from 0.25 to 2.0 mM, to blood prior to incubation with BAA, resulted in a time- and concentration-dependent attenuation of swelling, ATP depletion, and hemolysis of erythrocytes. Incubation of erythrocytes with BAA in calcium-free media or addition of EGTA had no effect on BAA activity.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21119-02 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism and Genotoxicity of Mutagenic Noncarcinogens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael L. Cunningham Senior Staff Fellow ETB NIEHS

Others: H.B. Matthews Research Chemist ETB NIEHS  
L. T. Burka Research Chemist ETB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

1.2

## OTHER:

0.3

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The purpose of the research in this laboratory is to investigate chemical and biochemical factors which account for 'false positive' mutagenic noncarcinogens. This investigation of nonconcordance between the short-term genotoxicity assays and the results of bioassays has determined that the mutagenic carcinogen-noncarcinogen pair 2,4- & 2,6-diaminotoluene (DAT) differ only in that the carcinogenic isomer produced a dose-related increase in cell proliferation in the liver whereas the noncarcinogen produced no increase in cell proliferation even at higher doses. Subsequent studies utilizing the incorporation of bromodeoxyuridine into newly synthesized DNA have demonstrated that the mutagenic noncarcinogen-carcinogen pair 1- & 2-nitropropane (NP) have a similar pattern of toxicity. That is, the hepatocarcinogenic isomer 2-NP produced a dose-related increase in cell proliferation in the liver yet the noncarcinogenic isomer 1-NP did not. These results suggest and further confirm a positive correlation between cell proliferation and carcinogenicity for these chemicals, irrespective of their mutagenicity. Future research will expand these findings with other chemical pairs, especially mutagenic carcinogen-noncarcinogen pairs whenever possible to further evaluate the role of cell proliferation in experimental carcinogenesis.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-ES-21123-02 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of Absorption of Chemicals Physically Bound to Feed By F344 Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	C.W. Jameson	Chemist	ETB	NIEHS
Others:	T.J. Goehl	Chemist	CCB	NIEHS
	B.J. Collins	Chemist	STB	NIEHS
	J. Yuan	Visiting Fellow	ETB	NIEHS

COOPERATING UNITS (if any)

Chemical Carcinogenesis Branch  
Systems Toxicity Branch

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Some chemicals physically bind to rodent feed. This binding usually increases with time. This phenomenon may also have an effect on the absorption of the chemical after ingestion by rodents. The objective of these studies is to determine if there is a difference in absorption of study chemicals in rats given freshly prepared vs. aged chemical feed mixes.

In an earlier NTP study the physical binding of o-nitroanisole (ONA) to NIH-07 rodent feed was found to increase with storage time. To evaluate this phenomenon, the systemic availability of ONA from aged and fresh feed formulations was determined by following the urinary excretion of ONA metabolites in male F344 rats using a crossover design. Prior to conducting the bioavailability study, analytical methods for the determination of ONA in feed and its major metabolite, o-nitrophenol (free and conjugated), in rat urine were validated. The results of this investigation indicated that there was no difference in the systemic availability of o-nitroanisole from fresh or aged feed formulations and therefore the physical binding of ONA to NIH-07 rodent feed had no effect on the absorption of the chemical after ingestion by F344 rats.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bile Acids as Indicators and Initiators of Hepatotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Morrow B. Thompson Vet. Med. Officer ETB NIEHS

Others: B.J. Bryant-Varela Research Technician ETB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Clinical Pathology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.2

## PROFESSIONAL:

## OTHER:

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Preliminary analysis of clinical chemistry results from 25 subchronic studies performed for the NTP has revealed that measurement of total bile acid concentration in the serum of rats is a sensitive and specific indicator of decreased hepatobiliary function. Although increased concentrations of bile acids frequently correlate with increased activities of hepatocellular enzymes in serum, in many studies, changes in concentrations of bile acids are often the only indication of treatment-induced hepatobiliary disease. For several years, we have been exploring the theory that alterations in concentrations of individual bile acids in laboratory animals can be used to identify specific lesions in the enterohepatic circulation and may initiate certain types of hepatic damage. A HPLC/enzymatic assay that permits the identification and quantitation of picomolar concentrations of individual bile acids in biologic samples has been developed in-house. In initial investigations in male and female rats in which 12 separate treatments were used (selection was based on the ability of each to disrupt a selected component of the enterohepatic circulation), analysis of the concentrations of individual bile acids permitted the correct identification of the treatment an animal received with approximately 95% accuracy. In 1990, studies were completed which demonstrated the promotional activity of a primary bile acid, chenodeoxycholic acid, on hepatocellular foci in the rat and which identified specific changes in the bile acid profile of rats treated with a peroxisome proliferator. The identification of an unknown bile acid, which becomes the predominant form in rats with a specific type of liver disease, is progressing through use of liver slice studies, enzyme analyses, and NMR and mass spectroscopy. In other efforts, bile acid studies are being conducted using rat liver slices. Initial studies using this technique have resulted in the selection of methods, defined media, and incubation times. A series of in vitro experiments has been initiated to explore the effects of bile acids and hepatotoxic chemicals on hepatocellular and microsomal enzymes and on the metabolism of bile acids. Using a HPLC assay with a post-column enzymatic reaction, the bile acid profile in approximately 200 samples has been determined this fiscal year.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21138-01 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Comparative Metabolism and Disposition of Aliphatic Nitriles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Burhan I. Ghanayem Toxicologist DTRT NIEHS

Others: Idalia Sanchez Biol. Lab. Tech. DTRT NIEHS  
Chantal Wall Biol. Aid DTRT NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Chemical Disposition

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.1

## PROFESSIONAL:

0.05

## OTHER:

0.05

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Methacrylonitrile (MAN) was nominated for carcinogenicity testing by the NTP. Because of the lack of information on the metabolism and disposition of MAN and its structural similarity to the known carcinogen, acrylonitrile (AN), studies were designed to investigate MAN metabolism and disposition and to compare this information to that of AN. Preliminary studies indicate that MAN is rapidly absorbed after gavage administration of 1.15, 11.5, or 115 mg MAN/kg to male F344 rats. Preliminary evidence suggests that the majority of MAN derived radioactivity is eliminated in the exhaled air as CO<sub>2</sub> or as volatiles and in the urine. Further, apparent saturation of MAN metabolism is seen at the highest dose administered resulting in slower metabolism and elimination.





DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01-ES-21143-01 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicology Studies of Lead

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Dr. M.P. Dieter	Toxicologist	ETB	NIEHS
Others:	Dr. C.W. Jameson	Chemist	ETB	NIEHS
	Dr. M.R. Elwell	Vet. Pathologist	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because the current inhalation exposure standards for lead sulfide are 3-fold greater than for lead sulfide, these chemicals were nominated by NIOSH for testing. A workshop of scientific experts concluded that initial studies should address the comparative bioavailability of different types of lead ores and pure lead salts to determine the scope of any additional studies on lead toxicity that might be conducted by the inhalation route. A contract research project was initiated to compare the toxicity and bioavailability of lead oxide, lead sulfide, lead acetate, and a sample of lead ore concentrate from Alaska. The chemicals will be fed to rats for 30 days and their blood lead levels monitored weekly. At study termination blood and bone lead, and urinary aminolevulinic acid will be measured. In addition, in-house research studies will be conducted to evaluate the biochemical toxicity of the different lead compounds in the blood and brain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01-ES-21144-01 ETB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oximes Research Project

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Dr. Michael P. Dieter	Physiologist	ETB	NIEHS
Others:	Dr. C.W. Jameson	Chemist	ETB	NIEHS
	Dr. M.R. Elwell	Pathologist	ETB	NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Toxicology Branch

SECTION

General Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The oximes are used in a variety of applications that include intermediates for the production of chemicals and pesticides, blocking agents in the polymer industry, chelators in the metal industry, additives in fuels, dyes, etc., and as biocides for water and waste treatment. Representative compounds from this class of chemicals were nominated by NCI because their toxicity and potential carcinogenicity had not been well characterized. Cyclohexanone oxime and butanone oxime studies were initiated in a contract laboratory to evaluate toxicity in F344 rats and B6C3F1 mice after 14 day and 90 day exposure to the chemicals in the drinking water. Variables that will be measured include body weight, organ weights, clinical signs of toxicity, hematology plus methemoglobin, histopathology, a reproductive screen, and a genotoxicity screen. In-house research studies will be conducted with these two chemicals plus butanal oxime, with a focus on bone marrow toxicity, after the completion of comparative metabolism studies. In addition, preliminary investigations of the influence of cyclohexanone oxime on glycol alkyl ether toxicity are underway, because this oxime interferes with alcohol metabolism.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-ES-21145-01 ETB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of Subchronic Oral Toxicity of 4-Chloro- $\alpha,\alpha,\alpha$ -Trifluorotoluene

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	C.W. Jameson	Chemist	ETB	NIEHS
Others:	J. Yuan	Visiting Fellow	ETB	NIEHS
	T.J. Goeh1	Chemist	CCB	NIEHS
	M.R. Elwell	Vet. Pathologist	ETB	NIEHS
	M.B. Thompson	Vet. Pathologist	ETB	NIEHS

## COOPERATING UNITS (if any)

Chemical Carcinogenesis Branch

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

General Toxicology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

0.75

## PROFESSIONAL:

0.50

## OTHER:

0.25

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Corn oil has been associated with proliferative exocrine pancreatic lesions in rats. Because of this, alternative vehicles for gavage studies are being investigated. alpha-Cyclodextrin is being studied as an alternative to corn oil because of its ability to "complex" with organic molecules and enhance their water solubility and/or suspendability.

This study is designed to characterize the toxicity associated with a 14-day repeated exposure of 4-chloro- $\alpha,\alpha,\alpha$ -trifluorotoluene to F344 rats and B6C3F1 mice via gavage using corn oil and alpha-cyclodextrin as the gavage vehicles. This study allows for the determination of target organs, the no-effect level (where possible), differences in sensitivity between sexes and species, and the slope of the dose response curve. This study also allows for the determination of the utility of alpha-cyclodextran as a suitable gavage vehicle and an alternative to the use of corn oil. The information obtained from these studies will provide the basis for determining doses for future toxicity studies of this chemical.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Testing of Chemicals of Interest in Salmonella

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Errol Zeiger	Supervisory Microbiologist	ETB	NIEHS
Others:	Dennis Pagano	Microbiologist	ETB	NIEHS
	Amal Abu-Shakra	Visiting Fellow	ETB	NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Experimental Toxicology Branch

## SECTION

Mutagenesis Group

## INSTITUTE AND LOCATION

NIEHS, Research Triangle Park, NC

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.5

## OTHER:

0.5

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The hair dye component, HC Blue 1 was subjected to HPLC to isolate impurities that may be responsible for its mutagenicity. The commercial dye and its mutagenic fraction, but not the HPLC-purified dye, produce DNA adducts in Salmonella. These results show that the mutagenicity of HC Blue 1 is due to its impurities.

The food mutagens, IQ and MeIQ, were studied following activation by S9 from different rat strains in the presence of biogenic amines. Tryptamine enhanced IQ mutagenicity by Fischer rat S9 and inhibited it with Sprague-Dawley and Wistar rat S9. Tryptamine enhanced the mutagenicity of MeIQ, regardless of the S9 source. Tyramine produced different patterns, and histamine had no effect. These patterns of interactions suggest rat strain differences in the activation of IQ and MeIQ.

Preincubation of Salmonella and metal salts in distilled-deionized water resulted in the detection of mutagenic activity with cobalt chloride, ferrous sulphate, cadmium chloride, and zinc chloride; phosphate buffer, which is normally used, reduces or eliminates the mutagenicity. Magnesium and citrate in the minimal medium were also responsible for these effects. HEPES buffer does not inhibit mutagenicity and may be a good alternative to phosphate for use with bacterial and mammalian cells.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01 ES 21009-09 STB</b>	
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Reproductive Effects in Males Exposed to Environmental Chemicals</b>			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	Jacqueline Williams	Visiting Fellow	STB      NIEHS
Other:	Kimberley Treinen Warren W. Ku	Staff Fellow IRTA	STB      NIEHS STB      NIEHS
COOPERATING UNITS (if any)			
Program Resources Group, DTRT Comparative Medicine Branch, NIEHS			
LAB/BRANCH Systems Toxicity Branch			
SECTION Developmental and Reproductive Toxicology Group			
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709			
TOTAL MAN-YEARS:		PROFESSIONAL:	
<b>1.3</b>		<b>1.3</b>	
		OTHER:	
		<b>0</b>	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither			
<input type="checkbox"/> (a1) Minors			
<input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)			
<p>Numerous chemicals encountered in the environment may alter reproductive functions. The rabbit studies ended, after showing that rabbits are less sensitive than humans to the male reproductive toxicity of ethylene dibromide. Additionally, there were no paternally-mediated teratologic effects, as shown by mating, nor was the function of a known number of sperm altered by EDB exposure.</p> <p>Additional studies this year have focused on further characterizing the lesion produced in the testis by boric acid. Previous studies suggested a hormonal component to the testicular atrophy seen after consumption of BA in the feed at 9000 ppm. Studies this year have further evaluated this; initial results from ongoing studies suggest only a small androgenic component. Additionally, we are evaluating the potential involvement of other neuroendocrine-dependent hormonal systems (thyroid, adrenal). Generally, no significant effects have been seen on these other hormone systems. The disposition of BA in selected target and non-target tissues is being determined. The potential of BA to cause in vivo riboflavin deficiency as a mechanism of the testicular toxicity is being investigated, as are the direct effects of BA applied to Sertoli or Leydig cells in primary culture from naive rats. Earlier NIEHS studies found elevated sperm acidic epididymal glycoprotein (AEG) levels; epididymal histology and serum AEG levels, as well as a semi-quantitative immunocytochemistry of AEG will further evaluate this initial finding. Future studies will evaluate the potential hormonal component in the testicular lesion caused by BA at lower doses (2000 ppm), and will compare the lesion development at these two dose rates (9000 vs. 2000).</p>			



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21031-06 STB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Computer Simulation of Inhalation Exposures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: M.P. Moorman Engineer STB NIEHS

Others: R. A. Sloane Biologist STB NIEHS  
R. S. Yang Research Chemist STB NIEHS

## COOPERATING UNITS (if any)

NSI-ES

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Respiratory Toxicology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.6

## PROFESSIONAL:

0.5

## OTHER:

0.1

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Computer simulations of physiologically based pharmacokinetic models are being used to study the uptake and metabolism of compounds administered by inhalation. The application of these models to specific compounds requires two types of compound specific data: tissue partition coefficients and metabolic constants. Systems for making these measurements have been developed by adapting the designs used by other laboratories. Tissue partition coefficients are determined by measuring the partitioning between tissue homogenates and the headspace in sealed vials. Metabolic rate constants are measured by monitoring the removal by the test animals of the compound from the atmosphere of a sealed recirculating exposure system. A computer simulation of the test animals and the exposure system is used to estimate the metabolic rates from these measurements. The values of metabolic parameters are determined by adjusting the metabolic constants of the simulation until the simulation results agree with the measured data. This method has been used to make in vivo measurements of the metabolism of test compounds in animals of different ages.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21046-07 STB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postnatal Toxicology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frank Kari Toxicologist

STB

NIEHS

Others: Bernard A. Schwetz Chief, STB

STB

NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Developmental &amp; Reproductive Toxicology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.6

## PROFESSIONAL:

0.6

## OTHER:

1

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

- 1) To evaluate the lactating rodent as a test system for assessing the potential of xenobiotics to concentrate in human milk.
- 2) To investigate the ability of xenobiotics to qualitatively alter the composition of milk

## Rationale and background:

It is well-known that neonates can be exposed to drugs/chemicals (and their metabolites) indirectly by transfer of the compounds through milk. A variety of medicinals have been evaluated for their propensity to travel in human milk and physico-chemical relationships have been established which allow predictions for other chemicals. However, humans are exposed to a large number of industrial, environmental, and agricultural chemicals for which no milk data are available. Therefore, a focus of this project has been to evaluate the lactating rodent as a model for testing the potential lactational transport of chemicals for which human studies are unfeasible. Additionally, the effects of chemicals on the lactation process is a neglected area of study which may have important implications in the development of neonates. Consequently we are investigating the ability of toxicants to alter milk quality and milk quantity.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>Z01-ES-21089-04 STB</b>
PERIOD COVERED <b>October 1, 1989 to September 30, 1990</b>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <b>Mechanism of Action of Testicular Toxicants</b>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Co-PI: Jerrold J. Heindel Co-PI: Robert E. Chapin	Expert Toxicologist	STB STB  NIEHS NIEHS
COOPERATING UNITS (if any)  Program Resources Group, DTRT Comparative Medicine Branch, DIR		
LAB/BRANCH Systems Toxicity Branch		
SECTION Developmental and Reproductive Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, NC		
TOTAL MAN-YEARS: <b>1.6</b>	PROFESSIONAL: <b>0.4</b>	OTHER: <b>1.2</b>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>           Various environmental and industrial chemicals can perturb male reproductive function. The objectives of these studies are to define subcellular target sites in testicular somatic cells in primary culture. For FY90, efforts have focused on effects of mono(2-ethylhexyl)phthalate, and the active metabolite of tri-o-cresyl phosphate (TOCP), saligenin cyclic o-tolyl phosphate, on Sertoli cells in primary culture. Efforts with saligenin have focused on identifying molecular changes in exposed Sertoli cells. Gel electrophoresis and phosphorylation studies are underway to examine changes in phosphorylation patterns seen after exposure to saligenin. With regard to MEHP, we have shown that it not only specifically inhibits FSH-induced cAMP accumulation in cultured Sertoli cells but that it has a site of action distal to cAMP formation since it inhibits 8 bromo cAMP induced estradiol secretion. We have also found that the action of MEHP on cultured Sertoli cell cAMP accumulation, in contrast to its action in vivo, are not age-dependent. Thus, further studies are needed to define the site and mechanism of action of MEHP on testicular function.         </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Arsenic Gas and Gallium Arsenide Toxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Daniel L. Morgan Toxicologist STB NIEHS

Others: B.A. Schwetz	Supervisory Pharmacologist	STB NIEHS
M.P. Moorman	Engineer	STB NIEHS
G.J. Rosenthal	Microbiologist	STB NIEHS
R.A. Sloane	Biologist	STB NIEHS
P.L. Blair	Supervisory Biologist	CCB NIEHS

## COOPERATING UNITS (if any)

NSI-ES  
University of Maryland

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Respiratory Toxicology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.05

## PROFESSIONAL:

0.05

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been conducted in the two previous years to evaluate the acute and short-term toxicity of arsine gas. Fischer 344 rats, B6C3F1 and C57BL/6 mice, and Syrian golden hamsters have been exposed to arsine gas at concentrations of from 10 ppb to 50 ppm for periods ranging from .5 hours to 90 days. All groups exposed to a single 6 hour exposure of 25 ppm experienced 100% mortality while those exposed to 5 ppm for four weeks or 2.5 ppm for 13 weeks showed no overt signs of toxicity. Urine samples from these studies showed increased levels of coproporphyrin and 7 and 8 carboxyl uroporphyrin. This data suggests that alterations in the heme biosynthetic pathway as reflected in increases of specific species of urinary porphyrins may be used as early biological indicators of ongoing arsine toxicity. A method has been developed to improve the measurement of specific porphyrin species in rodent urine to further refine this model. Tissues from these exposures are being analyzed for arsenic content to provide a measure of the tissue dose for specific exposure regimens.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 ES 21105-03 STB

PERIOD COVERED

October 1, 1989 to September 30, 1990 "TERMINATED" December 1, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Heat Shock Proteins in Testicular Somatic Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-PI: Robert E. Chapin

Toxicologist

STB

NIEHS

Co-PI: Randy L. Allen

Staff Fellow

LRDT

NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Developmental and Reproductive Toxicology Group

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21110-03 STB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Immunotoxicity Studies of Inbred Mice

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Jerrold J. Heindel Toxicologist  
Other: Steven D. Holladay IRTA

STB NIEHS  
STB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Developmental and Reproductive Toxicology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.1

## PROFESSIONAL:

1.1

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and naturally occurring toxins such as the tricothecene mycotoxin, T2 toxin, adversely affect immunologic function in offspring following treatment of pregnant mice over various periods of gestation.

Studies are in progress to explore the relationship between developmental immunotoxicity and the induction of structural malformations. These studies are, in particular, addressing the following question: Do modulations in lymphocytic surface antigens, induced by prenatal chemical exposure, result in functional immunologic deficits later in life?

TCDD or T2 toxin are administered to pregnant C57B1/6 and B6C3F<sub>1</sub> mice during gestation to establish the sensitive period for induction of immunologic deficits and to identify the initial lesion. Fetal T and B lymphocytes from the spleen and thymus, and subpopulations of these cells, are stained immunocytochemically to determine the morphological effects of TCDD and T2 toxin on lymphocytic surface antigens and their development. Fetal thymic organ culture is used to determine effects of exposure on subpopulations of immune cells. In addition, cell populations are evaluated by flow cytometry to determine quantitative changes. If changes in these surface markers persist beyond the age of 4 weeks in the prenatally exposed animal, functional tests, including mixed lymphocyte reaction, plaque assay, *in vitro* blastogenesis, cytotoxic T lymphocyte function, and colony forming unit assay, are conducted.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21116-02 STB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

## TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Primary Culture of Mixed Testicular Cells

## PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert E. Chapin Toxicologist

STB

NIEHS

Others: Jerrold J. Heindel Expert

STB

NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Developmental and Reproductive Toxicology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS:

1.0

## PROFESSIONAL:

0.3

## OTHER:

1.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No activity.



<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  Z01 ES 21118-02 STB
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Mechanism of Action of Ovarian Toxicants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI: Jerrold J. Heindel      Expert	STB	NIEHS
Others: Kimberley A. Treinen      Staff Fellow	STB	NIEHS
COOPERATING UNITS (if any)  Program Resources Group, DTRT Comparative Medicine Branch, DIR		
LAB/BRANCH Systems Toxicity Branch		
SECTION Developmental and Reproductive Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: <div style="text-align: right;">1.1</div>	PROFESSIONAL: <div style="text-align: right;">1.1</div>	OTHER: <div style="text-align: right;">0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>             Mono(2-ethylhexyl)phthalate (MEHP) is both a male and female reproductive toxicant as determined in the NTP Reproductive Assessment by Continuous Breeding protocol. In the male, MEHP has been shown <i>in vivo</i> and <i>in vitro</i> to be a Sertoli cell (SC) toxicant. <u>In vitro</u> MEHP inhibited FSH-stimulated cAMP accumulation in cultured SCs. This inhibition occurred after a 6 hr preincubation period, with maximal inhibition (50%) by 24 hrs. Half-maximal inhibition is seen at 12-15 <math>\mu</math>M MEHP. Since MEHP is also a female reproductive toxicant, and granulosa cells are thought to be the female counterpart to SCs, we examined the effect of MEHP on FSH-stimulated cAMP accumulation in cultured granulosa cells (GCs). GCs are harvested by ovarian puncture of DES-primed immature (19-22 d) F-344 rats and 300,000 viable cells were incubated in plastic tubes for up to four days. FSH, forskolin, and isoproterenol were shown to stimulate cAMP accumulation. MEHP inhibited FSH-stimulated cAMP accumulation in a dose- and time-dependent manner. Significant inhibition (30-50%) of GC cAMP accumulation occurred with 200 <math>\mu</math>M MEHP after a 15 hr exposure, with maximal inhibition at 30 hrs. MEHP also inhibits progesterone production. Thus, the action of MEHP on GCs appears to be limited to an effect to decrease FSH-stimulated cAMP production. This decrease in cAMP levels then results in decreased progesterone production which may be an important part of the female reproductive toxicity of MEHP.           </p>		



## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 ES 21124-02 STB

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Development of a Model to Study the Influence of Nutrition on F344/N Rat Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frank W. Kari  
Others: J. E. FrenchToxicologist  
PhysiologistSTB NIEHS  
ECMB NIEHS

## COOPERATING UNITS (if any)

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Developmental &amp; Reproductive Toxicology Group

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

## TOTAL MAN-YEARS

0.2

## PROFESSIONAL:

0.2

## OTHER:

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to characterize the role of nutritional and endocrine factors in the progression of mononuclear cell leukemia (MNCL) in Fischer 344/N (F344) rats, with emphasis placed on explaining documented idiosyncrasies in the occurrence of this tumor. We will achieve our goal by first testing the hypothesis that nutritional manipulations and experimental-induced alterations in selected hormones/growth factors influence the incidence and latency period of MNCL in F344 rats using a short-term in vivo transplant model. These whole animal treatments will be further characterized for their ability to alter cellular proliferation and differentiation of MNCL cells using a diffusion chamber system in situ. Our experiments have been designed to explain idiosyncrasies in spontaneous MNCL incidence, including gender differences in background incidence and in response to corn oil gavage. Successful completion of our goal will provide guidance for reducing variability and decreasing the spontaneous incidence of MNCL in F344 rats. This will lead to increased statistical power, reduced confounding and clarified interpretation of carcinogen bioassay data used in risk assessment.

Our long-term objective is to establish well-defined laboratory models for evaluating chemical, nutritional and endocrine modulations on leukemia progression. This work represents a collaborative effort between nutritional scientists at the University of North Carolina and toxicologists at the National Institute of Environmental Health Sciences.





<b>DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE</b> <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>		PROJECT NUMBER  <b>201 ES 21127-01 STB</b>
PERIOD COVERED <u>October 1, 1989 to September 30, 1990</u>		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) <u>Short-term Comprehensive Reproductive and Developmental Toxicity Screen</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Co. PI: Robert E. Chapin	Toxicologist	STB NIEHS
Co. PI: Martha W. Harris	Biologist	STB NIEHS
COOPERATING UNITS (if any) Program Resources Group, DTRT Comparative Medicine Branch, DIR Chemical Carcinogenesis Branch, DTRT		
LAB/BRANCH Systems Toxicity Branch		
SECTION Developmental and Reproductive Toxicology Group		
INSTITUTE AND LOCATION NIEHS, NIH, Research Triangle Park, North Carolina 27709		
TOTAL MAN-YEARS: 1.7	PROFESSIONAL: 0.2	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)  <p>Assays for reproductive toxicity have heretofore been long and cumbersome, necessitating months of dosing to assure sufficient exposure of potential germ cell targets. However, the number of chemicals for which no reproductive or teratologic data exist is large, and growing larger. What is needed is a test that would help prioritize chemicals for complete reproductive testing. This design uses one group of 10 male mice, which are mated before treatment to a group of 10 females/dose group. These females are dosed during gestational days 8-14, are allowed to give birth, and nurse the young until day 4. The males are then dosed with the test compound, and mated, after 4 days, with another group of females for 5 days. Both males and females are dosed during mating and up until sacrifice. These females are sacrificed on g.d. 10-15, and uterine contents assessed. The males are sacrificed after 19 days of exposure, and subject to a thorough reproductive necropsy (epididymal sperm evaluations, organ weights, and histology). Because this design evaluates many aspects of reproductive and development both functionally (sperm motility, fertilizing capability, ovulation, implantation, development and nursing) and structurally (detailed histopath on potential target organs), it is expected to identify those compounds that possess significant reproductive and/or teratologic activity. Tests are currently underway to assess the sensitivity of this design, using chemicals of known potency (generated from previous Continuous Breeding studies).</p>		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
201 ES 21135-01 STB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effects of AIDS Therapeutics on the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gary J. Rosenthal Toxicologist STB NIEHS

Others: M. Luster Research Microbiologist STB NIEHS  
B. Blaylock IRTA STB NIEHS

COOPERATING UNITS (if any)

LAB/BRANCH

Systems Toxicity Branch

SECTION

Immunotoxicology Section

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, North Carolina 27709

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The Immunotoxicology Group has initiated the assessment of immunotoxicity of AIDS therapeutics. The ongoing objectives include: (1) to evaluate the potential adverse effects of promising AIDS therapeutics on systemic as well as local systems; (2) if possible, to examine potential mechanisms of toxicity (or therapeutic action, if unknown); (3) to relate these observed changes in immune function to clinicians and regulatory agencies so that improved treatment and monitoring may be facilitated.

Studies were performed in the following areas: a) Descriptive immunotoxicity studies on pentamidine isethionate and alpha-interferon on pulmonary macrophages. The endpoints for these studies being cytokine production, antigen presentation, phagocytosis, and reactive oxygen intermediate production, and NK cell mediated tumor killing; b) Mechanistic studies on the cellular and subcellular targets of these drugs; and c) Immunopharmacologic examinations of pentamidine and alpha-interferon in disease models.

The results of these studies demonstrated an inhibition of cytokine production by pentamidine, most notably interleukin 1. This inhibition was shown to be mediated via a post-translational protein modification event. Consistent with this, pentamidine protected mice from endotoxin shock and inhibited the hypersensitivity response to the sensitizer, oxazolone. With respect to alpha-interferon, pulmonary macrophages were specifically stimulated functionally by the drug. This correlated with increased resistance to pulmonary melanoma metastasis and marginally with pulmonary influenza.



## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicity Studies of Styrene and Related Chemicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Daniel L. Morgan Toxicologist STB NIEHS

Others: B.A. Schwetz Supervisory Pharmacologist STB NIEHS  
M.P. Moorman Engineer STB NIEHS

## COOPERATING UNITS (if any)

NSI-ES

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Respiratory Toxicology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

0.25

## PROFESSIONAL:

0.25

## OTHER:

0

## CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unrevoked type. Do not exceed the space provided.)

Inhalation toxicity studies were designed to study the toxicity and potential carcinogenicity of styrene and two congeners, alpha-methylstyrene and divinylbenzene. The research proposal has been prepared and reviewed and final protocols are being written. Studies on styrene will be initiated in September-October 1990. Studies will include evaluation of cell turnover in the respiratory tract and liver of rats and mice, the leukemia transplantation test in rats, and evaluation of micronuclei and immunotoxicity in mice. These studies are designed to provide scientific information on the comparative toxicity of styrene and its congeners and on possible mechanisms for potential carcinogenicity of these important chemicals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 ES 21137-01 STB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Tetranitromethane Toxicity and Carcinogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Daniel L. Morgan Toxicologist STB NIEHS

Others: B.A. Schwetz Supervisory Pharmacologist STB NIEHS  
M.P. Moorman Engineer STB NIEHS

COOPERATING UNITS (if any)

NSI-ES

LAB/BRANCH

Systems Toxicity Branch

SECTION

Respiratory Toxicology

INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A research proposal was submitted for investigating the mechanism(s) of tetranitromethane (TNM) pulmonary toxicity and carcinogenicity. This project will include studies of the reactions of TNM with cellular DNA and proteins, pharmacokinetics and metabolism, comparison of nasal and alveolar carcinogenicity, and comparison with related nitro-substituted hydrocarbons. The proposal has been reviewed and accepted by internal and external reviewers. Supplies and equipment are being acquired for initial studies investigating the biochemical reactions of TNM with purified nucleosides and DNA.





## NOTICE OF INTRAMURAL RESEARCH PROJECT

## PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toxicology of Environmental Chemicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: B.A. Schwetz Supervisory Pharmacologist STB NIEHS

Others: M.P. Moorman Engineer STB NIEHS

R.A. Sloane Biologist STB NIEHS

G.J. Rosenthal Microbiologist STB NIEHS

M.P. Dieter Physiologist STB NIEHS

## COOPERATING UNITS (# any)

NSI-ES

## LAB/BRANCH

Systems Toxicity Branch

## SECTION

Respiratory Toxicology

## INSTITUTE AND LOCATION

NIEHS, NIH, Research Triangle Park, NC 27709

## TOTAL MAN-YEARS:

9

## PROFESSIONAL:

5

## OTHER:

0.4

## CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

## SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work has continued on refining the technology involved in studying the toxic effects of compounds administered by inhalation. These technological capabilities are critical to conducting inhalation studies accurately and safely. Significant advances have been made in controlling the exposure atmosphere, in documenting environmental conditions, in collecting and standardizing in-life observations, and in managing the data resulting from inhalation studies. Test animals are identified with implanted microchip transponders which are read automatically by the data system. The ability to control and accurately monitor all aspects of actual exposure has allowed studies of extremely toxic compounds, such as arsine, to be conducted safely. A two-year chronic exposure to methylene chloride is being conducted to investigate cellular and molecular processes responsible for the induction of lung and liver tumors. A study of three structurally-related compounds--styrene, alpha-methylstyrene, and divinylbenzene--will investigate leukemogenic potential and effects on pulmonary function.



**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE  
NOTICE OF INTRAMURAL RESEARCH PROJECT**

**PROJECT NUMBER**  
Z01 ES 30106-16 STB

**PERIOD COVERED**

October 1, 1989 to September 30, 1990

*TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)*

The Effects of Environmental Pollutants on the Immune System

*PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Michael I. Luster	Research Microbiologist	STB NIEHS
Others:	M. Taylor	Staff Fellow	STB NIEHS
	G. Rosenthal	Toxicologist	STB NIEHS
	B. Blaylock	IRTA	STB NIEHS
	J. Heindel	Expert	STB NIEHS
	S. Holladay	IRTA	STB NIEHS

**COOPERATING UNITS (if any)**

**LAB/BRANCH**

Systems Toxicity Branch

**SECTION**

Immunotoxicology Section

**INSTITUTE AND LOCATION**

NIEHS, NIH, Research Triangle Park, North Carolina 27709

**TOTAL MAN-YEARS:**

6.0

**PROFESSIONAL:**

3.0

**OTHER:**

3.0

**CHECK APPROPRIATE BOX(ES)**

☐ (a) Human subjects      ☐ (b) Human tissues      ☒ (c) Neither  
☐ (a1) Minors  
☐ (a2) Interviews

**SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)**

The Immunotoxicology Group studies the adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and biological materials. The ongoing objectives include efforts: (1) to evaluate and examine the influence of selected drugs or environmental chemicals on the immune response and relate alterations in immunological functions with general and specific organ toxicity; (2) when applicable, to examine potential mechanism of action; (3) to relate changes in immunological functions with altered host resistance following challenge with tumor cells or infectious agents; and (4) to refine and validate a panel of immune and host resistance procedures in order to better define immunotoxicity and correlate changes in immune function with altered host resistance. General methodology employed includes various cell and tissue culture procedures, flow cytometry, electrophoresis (northern and western blots), hematological procedures and biochemical tests to determine the activity of immune cells. Studies have been conducted in the following areas: (a) Development and utilization of procedures to examine the effects of in utero exposure to selected environmental chemicals on the developing immune system in mice and, in particular, thymic maturation; (b) Development and utilization of model systems which allow assessment of specific macrophage populations [i.e., alveolar (lung) and Langerhans (skin) cells]. Endpoints for these assessments include production of soluble mediators (monokines), surface markers and effector cell function; (c) Examination of the mechanisms of chemical-induced thymic atrophy with particular emphasis on apoptotic mechanisms (programmed cell death); (d) Examining the control of cytochrome P450c and the TCDD-inducible or tumor associated aldehyde dehydrogenase in selected rat immune cell and lymphoid organs; (e) Examining the mechanism(s) associated with increased endotoxin sensitivity following exposure to selected compounds including T-2 toxin and TCDD.

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